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MINIREVIEW

Regulation of Apoptosis by Viral Gene Products

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Apoptosis is the process whereby individual cells of multicellular organisms undergo systematic self-destruction in response to a wide variety of stimuli. Apoptosis is a genetically controlled preprogrammed event which eliminates cells during development when they have become redundant or which functions as an emergency response after radiation damage, viral infection, or aberrant growth induced by the activation of oncogenes. In the case of virus-infected cells, the induction of early cell death would severely limit virus production and reduce or eliminate spread of progeny virus in the host. Thus, most animal viruses have evolved strategies to evade or delay early apoptosis to allow production of high yields of progeny virus. Over the past few years both the biochemical basis of apoptosis and its regulation by viral products have become clearer. For example, part of the apoptotic program includes the induction of cellular endonucleases which could target replicating viral DNA and prevent virus production at a very early stage. Thus, the problem facing the virus is to replicate and package large numbers of progeny genomes safely within newly synthesized viral capsids. Many viruses have evolved genes encoding proteins which effectively suppress or delay apoptosis long enough for the production of sufficient quantities of progeny. In addition, a growing number of viruses are now known to induce apoptosis actively at late stages of infection. This process may represent a final and important step in the spread of progeny to neighboring cells while also evading host immune inflammatory responses and protecting progeny virus from host enzymes and antibodies. Such virally induced apoptosis may also contribute to some clinical manifestations and cytotoxicity associated with several human diseases of viral origin.

The purpose of this review is to summarize recent information on the induction and suppression of apoptosis by viral products as well as to propose how this knowledge may provide insights into basic cell biology and offer the potential of new therapeutic applications. A recent comprehensive review by Shen and Shenk (75) also deals with some aspects of the subjects covered below.

APOPTOSIS

The process of apoptosis can be divided into three stages. The first is the initial signal for apoptosis to commence and can be provided by a large variety of stimuli (reviewed in reference 79). In lymphoid cells, for example, the Fas receptor (CD95) is a key component. In a great many cells the p53 tumor suppressor plays a major role in response to genotoxic stresses such as X- and gamma-irradiation and expression of cellular or viral oncogenes. After receipt of an initial signal there usually follows a lag period of various lengths, depending on the specific stimulus and cell type. The second stage involves the execution phase in which cells undergo the classic morphological changes associated with apoptosis, including condensation of chromatin and vacuolization of the cytoplasm. At this stage a number of cellular proteases and endonucleases are activated and cellular DNA is degraded to characteristic nucleosome-sized fragments which migrate as a ladder on agarose gels. The final stage involves the formation of membrane-bound apoptotic bodies which are completely engulfed by surrounding cells through phagocytosis. As we will describe below and as is summarized in Fig. 1, viral proteins have been implicated in both the induction and suppression of apoptosis. A summary of some relevant viruses and gene products is presented in Tables 1 and 2.

CONTROL OF p53-INDUCED APOPTOSIS

Several viruses have developed specific strategies to block apoptosis induced by p53. In some cases these are highly necessary because induction of unscheduled DNA synthesis appears to be linked to the activation of p53 and p53-dependent apoptosis. Small DNA tumor viruses all share the common property of being able to induce DNA synthesis in quiescent host cells. This function is essential because all must activate host cell DNA synthetic machinery to replicate viral DNA. Human adenoviruses (Ad), simian virus 40 (SV40), polyomavirus, and human papillomaviruses (HPV) have been the best characterized. Each encodes proteins that interact with key regulators of the cell cycle to stimulate unscheduled DNA synthesis. The effect of these interactions also is to activate the stabilization and/or accumulation of the p53 tumor suppressor and the induction of growth arrest or apoptosis in a p53-dependent manner. The early region 1A (E1A) product of Ad increases p53 levels and induces p53-dependent apoptosis (21, 50). Expression of the SV40 large T antigen leads to an increase in p53 levels; however, T antigen also binds to and inactivates p53. Several groups have demonstrated with transgenic mouse models that expression of mutant forms of T antigen which are unable to bind to p53 results in p53-dependent apoptosis (29, 54). Expression of the E7 protein of HPV also activates both p53-dependent and p53-independent cell death (64). A common property shared by all of these viral oncoproteins is the ability to interact with the retinoblastoma tumor suppressor protein Rb which regulates transcription factor E2F and thus cell cycle progression. Although abrogation

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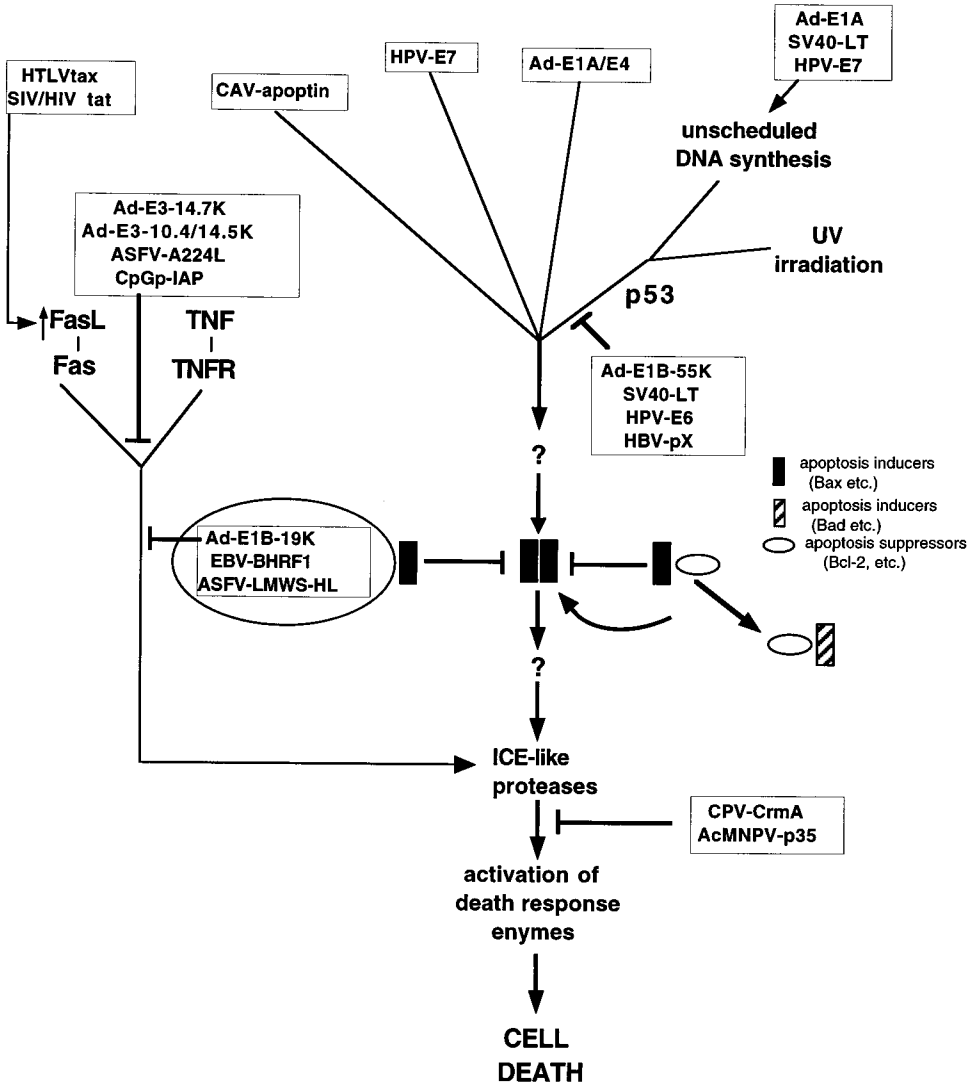

FIG. 1. Viral proteins affecting the apoptotic pathway. The apoptosis pathway has been illustrated. Viral proteins have been indicated in the boxes. Cellular apoptosis inducers such as Bax (solid rectangles) or Bad (hatched rectangles) have been indicated as has the Bcl-2 family of suppressers (open ovals).

of Rb may be a major cause of the activation of p53 and cell death in response to these viral products, there is evidence, at least in the case of E1A, that complex formation with the p300 transcriptional modulator and related proteins may be of equal or more importance (58, 66).

The mechanism by which p53 induces apoptosis is still unresolved. p53 is a transcription factor which contains both sequence-specific DNA binding activity and an independent acidic activation domain. The activation domain is probably critical for induction of apoptosis (70), although some studies have argued that it may not be essential (8, 39). One cellular target for p53 activation appears to be *bax* which encodes a downstream regulator of apoptosis (56). Each of the small DNA tumor viruses has adopted a different strategy to cope with the lethal effects of p53. SV40 large T antigen binds directly to the p53 DNA binding region and blocks interactions with p53-specific promoter elements (3). The early region 1B (E1B) 55-kDa protein (E1B-55K) of Ad5 binds to the activation domain of p53, thus tethering its transcriptional repression domain to p53 and blocking activation of p53-responsive

TABLE 1. Viruses and viral proteins implicated in inducing apoptosis

Virus	Protein(s) involved	Reference(s)
Ad	E1A	21, 50
	E4	53
	E3	90
SV40	Large T	29, 54
HPV	E7	64
Human T-cell leukemia virus type 1	Tax	99
CAV	Apoptin (VP3)	43
Sindbis virus	ND[a]	46
Baculovirus	ND	16
SIV	Tat	1
Parvovirus	NSP	57
PRRS	ORF 5 (p25)	80
HIV-1	Tat	48

[a] ND, the virus has been observed to induce apoptosis in the course of infection, but the viral protein(s) inducing this effect has not been determined.

TABLE 2. Viruses encoding apoptosis-inhibiting proteins

Virus	Protein(s) involved	Reference(s)
Ad	E1B-19K, E1B-55K, E3-14.7K, E3-10.4K/14.5K	21, 97, 85, 31, 32
Human cytomegalovirus	IE1 and IE2	102
Cowpox virus	*crmA*	87
Vaccinia virus	SPI-2	23
Myxovirus	T2 and M11L	52
Baculovirus	p35	16
	iap	20
HSV	$\gamma_1$34.5 gene	15
Herpesvirus saimiri	ORF16	76
SV40	Large T	29, 54
African swine fever virus	LMW5-HL (Bcl-2 homolog), A224L (IAP homolog)	2, 59, 10
HBV	pX	93
EBV	BHRF1 and LMP 1	42
HPV	E6	63, 91

genes (100). E1B-55K has been shown to block p53-dependent apoptosis, and this function requires 55K repression activity (85). Recently a second Ad protein, E4orf6, has also been shown to bind to p53 and inhibit p53 transactivation activity (24). E1B-55K and E4orf6 appear to bind to opposite ends of the p53 molecule and thus may inhibit its function by different mechanisms. There is no evidence that E4orf6 possesses intrinsic repression activity; however, it may disrupt interactions between p53 and TAFII31 which are required for p53-mediated transactivation (51, 89). Very recently it has been reported that E4orf6 blocks p53-dependent apoptosis, possibly by reducing the overall level of p53 (56a). HPV has evolved yet another distinct mechanism. The E6 proteins of the high-risk group of HPV with respect to cervical carcinoma also bind p53, but this reaction marks p53 for degradation via the ubiquitin pathway (73). The specific role for E6 is believed to be as a bridge between p53 and E6-AP, a ubiquitin-protein ligase, thereby facilitating the rapid degradation of p53 (72).

Mouse polyomavirus does not appear to have developed a specific strategy against p53; however, two additional DNA viruses have. The hepatitis B virus (HBV) pX protein interacts with p53, diminishing both DNA binding and transactivation activities (92). pX plays an important role in HBV-induced hepatocellular carcinomas and is often the only viral protein expressed in such tumors. Presumably its function, like those of the small DNA tumor virus products, is to block p53-mediated cell death (93). Epstein-Barr virus (EBV) also encodes two proteins that bind to p53. One is EBNA-5 which appears to interact with both Rb and p53 (83) and may function to disrupt the normal cell cycle in a fashion similar to that of the DNA tumor virus oncoproteins described above. The other is BZLF1 which binds to the carboxy terminus of p53 and blocks transactivation (101). This interaction may parallel that of Ad E4orf6 which binds to a similar region of p53 (24). The specific role of BZLF1 may be to control the shift from latent to lytic infection which could be blocked by high levels of p53 (101). The evolution of such varied and widespread strategies clearly signals the importance of p53 in host cell defense against virus infection and the need for viruses to eliminate p53 as a functional cell product.

VIRAL INHIBITION OF APOPTOSIS

Several viral proteins appear to block apoptosis by interacting directly with elements of the highly conserved biochemical pathway which regulates cell death. As discussed above, p53 is an important trigger of apoptosis and is commonly activated following infection by many viruses. However, mounting evidence suggests that infection by many viruses can induce p53-independent apoptosis. It is becoming apparent that increasing numbers of viral proteins have evolved which function downstream of p53 in the common apoptotic pathway.

Human Ad. It was with human Ad that the first clear links between cytotoxicity of virus infection and apoptosis were made. Early studies indicated that some Ad mutants could induce a dramatic phenotype, termed *cyt*/*deg*, in which infected cells succumbed to rapid cytolysis, yielding large plaques and degradation of both viral and cellular DNA (25). This effect was shown to be apoptosis and was mapped to the E1B-19K protein (65, 81, 96). E1B-19K is now believed to be a member of a growing family of cellular and viral proteins which includes both inhibitors and accelerators of apoptosis, of which Bcl-2 is considered to be the prototype (reviewed in reference 95). Bcl-2 is able to complement 19K-defective Ad mutants both in the inhibition of the *cyt*/*deg* phenotype and in the formation of stably transformed rodent cells (13, 60, 67). Although E1B-19K and Bcl-2 share only very modest and limited sequence homology (BH domains), they have been observed to form complexes with a similar array of cellular proteins and are believed to be functionally analogous (6). Like Bcl-2, E1B-19K interacts via BH domains with cellular proteins of the Bcl-2 family, including Bax (11, 36) and Bak (26), which are believed to be positive regulators of apoptosis. E1B-19K also interacts via a BH3 motif with another death inducer of this family, Bik/Nbk (5, 37). Figure 1 shows that the Bcl-2 family of apoptosis inhibitors appears to function by heterodimerization with family members such as Bax, thus preventing the formation of apoptosis-promoting homodimers. An additional type of regulator exists, typified by Bad, which induces apoptosis by binding to Bcl-2, thus freeing Bax to form death-promoting homodimers. Interestingly, E1B-19K does not interact with Bad (11), and thus Ad-infected cells are resistant to Bad-mediated cell death.

At least three other viruses also encode Bcl-2 homologs. The LMW5-HL protein of African swine fever virus encodes a protein containing sequences homologous to Bcl-2 (59), and this product has been found to inhibit apoptosis (2). The BHRF1 protein of EBV contains a central region with extensive homology to Bcl-2 and has been shown to protect B cells from death by apoptosis (41). Herpesvirus saimiri also produces a Bcl-2 homolog (76). Thus, clearly a common viral strategy has been the evolution of BH-containing proteins which regulate the Bax-Bcl-2 setpoint to block apoptosis and promote survival of infected cells.

Both Ad E1B-19K and Bcl-2 have been shown to block p53-dependent apoptosis (12, 21). In addition to transcriptional activation, p53 also has been reported to repress transcription independently of sequence-specific DNA binding and both Bcl-2 and 19K block this function (69, 74). There is to date no compelling evidence to indicate that transcriptional repression by p53 is required for or involved in apoptosis, and thus it is unclear whether the lifting of this repression by E1B-19K and Bcl-2 is related to protection against cell death.

In addition to blocking E1A-induced apoptosis by the mechanism described above (67), E1B-19K also is able to suppress cell death mediated by tumor necrosis factor alpha (TNF) and Fas ligand (31, 38, 97). The molecular basis for this inhibition is unclear because, as illustrated in Fig. 1, recent evidence (reviewed in reference 28) has suggested that these agents induce apoptosis not via the Bcl-2-regulated setpoint, but rather by activating downstream ICE-like proteases directly (see below). Thus either 19K possesses additional functions, or

family members other than Bcl-2 can regulate ICE-like enzymes by an as-yet-undetermined mechanism. Despite the effectiveness of 19K in blocking apoptosis mediated by TNF, Ad encodes three additional polypeptides in the E3 transcription unit which are able to block TNF-induced apoptosis specifically (31, 32). The E3-14.7K protein and the E3-10.4K/14.5K heterodimer are able to prevent TNF-mediated apoptosis independently, possibly by blocking the TNF-induced release of arachidonic acid, thereby interfering with downstream signaling from the TNF receptor (45). More recently it has been shown that the inhibition by 10.4K/14.5K may relate to TNF-induced translocation of cytosolic phospholipase A2 to membranes (22). In addition, these E3 proteins have been found to inhibit Fas-induced apoptosis, possibly by enhancing clearing of Fas from the cell surface (98). It is likely that these functions promote virus infection in vivo by preventing apoptosis induced by cytotoxic T cells, thus allowing the virus to escape destruction by the host immune system.

Poxviruses. Yet another mechanism of inhibiting apoptosis has been observed with the cowpox virus *crmA* gene product. One of the major setpoints regulating the terminal steps of apoptosis involves activation of a series of cysteine proteases, of which interleukin-1β-converting enzyme (ICE) is the mammalian prototype. These proteases in turn activate a series of enzymes that rapidly kill the cell. The cowpox virus CrmA protein encodes a protease inhibitor of the serpin family which specifically inhibits members of the ICE family and prevents or delays apoptosis. Such protection by CrmA inhibits apoptosis caused by cytotoxic T cells (88) and Fas signaling (87). Another poxvirus, vaccinia virus, has also been shown to encode a protein highly homologous to CrmA which inhibits apoptosis induced by TNF-α or Fas (23). Products of a rabbit poxvirus, myxomavirus, also inhibit apoptosis (52). Infected cells undergo rapid apoptosis after infection with virus mutants, producing defective T2 or M11L proteins. T2 shares homology with the TNF receptor (TNFR) and thus may compete for binding of TNF. M11L is a novel transmembrane protein of unknown function.

Baculoviruses. The products of two viral genes have been implicated in the inhibition of apoptosis in baculovirus-infected cells. Mutations in the gene encoding the p35 protein of *Autographica californica* nuclear polyhedrosis virus (Ac*M*NPV) result in extensive induction of apoptosis and a reduction in infectivity both in vivo and in vitro (16, 17). A second class of baculovirus genes, *iap* (inhibitor of apoptosis), also prevents apoptosis. *Cydia pomonella* granulosis virus (CpGP) and *Orgyia pseudotsugata* polyhedrosis virus both encode IAP-like products which are able to block apoptosis induced by p35 mutants of Ac*M*NPV (20). Although Ac*M*NPV also encodes a homolog of IAP, it is unable to inhibit apoptosis caused by the loss of p35 (20). The p35 protein is believed to function much like CrmA of cowpox virus, acting as an inhibitor of ICE-like proteases (7). The mechanism by which IAP proteins inhibit apoptosis is not yet understood but they contain a conserved C3HC4 (RING) zinc finger at the carboxy terminus, thus implying a role in transcriptional regulation (20). Also conserved among the various IAP proteins is a double repeat amino acid sequence near the amino terminus which has now been termed the baculovirus *iap* repeat (BIR). Domain swaps between IAPs from Ac*M*NPV and CpGP have shown that both the zinc finger and BIR regions are essential for inhibition of apoptosis (18). Further underscoring the importance of the viral IAP genes, several mammalian and *Drosophila* homologs have now been isolated (40, 49, 68) and in every case these inhibitors of apoptosis each possess the RING finger and BIR motifs. Two of these cellular IAP proteins were cloned as a result of interactions with the TNFR-TRAF signaling complex and may represent downstream effectors in TNF or Fas pathways (68). A homolog of IAP has also been identified in African swine fever virus, but its potential to prevent apoptosis remains to be tested (10). Thus, another strategy of several viruses has been to develop proteins with homology to cellular polypeptides involved in TNF- and Fas-mediated signaling and regulation of ICE-like proteases.

HSV. The $\gamma_1$34.5 gene of herpes simplex virus type 1 (HSV-1) has been shown to be essential for effective replication and lethal infection in rodent brains (14). This gene is not required for viral replication in cell culture but has been shown to prevent shutoff of protein synthesis in human neuroblastoma cells which is associated with apoptosis in neurons (15). Such inhibition of the induction of apoptosis in infected neurons and promotion of neurovirulence could represent a basis for HSV-mediated encephalitis in humans.

EBV. Lytic infection of human lymphocytes by EBV is believed to induce apoptosis, as evidenced by the appearance of highly condensed chromatin and extensive formation of cytoplasmic vacuoles (44). The EBV BHRF1 protein has regions of strong homology with Bcl-2 (41) and may play a role in limiting apoptosis during infection much like E1B-19K of Ad (84). In addition, another EBV product, latent membrane protein 1 (LMP-1), has been shown to prevent apoptosis (33) and this effect may be due at least in part to its ability to induce expression of Bcl-2 (42). It may be that induction of apoptosis is a necessary event during late stages of infection and that inhibition of apoptosis may be an important step in the shift to a latent phase. In fact, the control of apoptosis by EBV products may resemble the situation with Sindbis virus in which shifts from latent to lytic phases seem to be dependent on the regulation of apoptosis (see below).

Cytomegalovirus. Two immediate-early gene products of human cytomegalovirus, IE1 and IE2, have recently been shown to inhibit apoptosis (102). IE1 and IE2 are transcription factors and have been demonstrated to possess both transcriptional repression and activation properties in a variety of systems, suggesting that these proteins may function either through the activation of genes encoding inhibitors of apoptosis or repression of those producing accelerators of cell death. IE2 has been shown to interact with Rb and also to bind to p53 and repress its transcriptional activity (35, 77, 78). Thus, the properties of this herpesvirus parallel those described above for some of the small DNA tumor virus oncoproteins.

VIRAL INDUCTION OF APOPTOSIS

One of the least studied aspects of apoptosis concerns the final steps in which the apoptotic cell becomes vacuolized and endocytosed by surrounding cells in situ. Rather than releasing the entire contents of apoptotic cells into the interstitial fluid, components of cells undergoing apoptosis are encased in membrane-bound apoptotic bodies which are neatly absorbed into neighboring cells. Thus, in the case of virus-infected cells, apoptosis represents a very efficient mechanism by which the virus can induce cell death and disseminate progeny while limiting induction of inflammatory and immune responses. The presence of progeny virions in membrane-bound bodies also protects them from contact with neutralizing antibodies. Although enveloped viruses generally exit infected cells by budding from the plasma membrane, there is no mechanism which explains how nonenveloped, nonlytic viruses exit the dying host cell. Growing evidence suggests that virus-induced apoptosis may play a key role.

CAV. The cytopathic effect displayed by chicken anemia virus (CAV)-infected cells both in vivo and in vitro greatly resembles apoptosis (43). The CAV VP3 protein, termed apoptin, is capable of inducing apoptosis when expressed independently (61) in a wide variety cell types by a process which is independent of p53 (103). Cells undergoing CAV-induced apoptosis display apoptotic bodies containing virus particles which are endocytosed by surrounding epithelial cells (43). Cells actively infected with Sindbis virus (see below) also display extensive blebbing of the cytoplasm and accumulation of virus particles within similar structures (30). These observations and others raise the possibility that at late stages of some viral replication cycles, the induction of apoptosis may be a favored and necessary event.

Human Ad. Ad may also encode proteins which function as inducers of apoptosis at later stages of infection. Cytoxicity of Ad was originally believed to originate from the E1A protein which induces accumulation of p53 and p53-dependent apoptosis. However, Ad has also been shown to induce apoptosis in the absence of p53 (82, 86) and this activity is dependent on one or more E4 products (53). The induction of both p53-dependent and p53-independent apoptosis may explain the need for two E1B proteins, E1B-55K, which prevents p53-mediated cell death, and E1B-19K, which blocks all forms of apoptosis. The identity of the E4 protein(s) involved and the mechanism of cell death are under study. In addition, an E3 product has recently been implicated in Ad-induced cell death (90). The E3-11.6K product, termed the Ad death protein (ADP), is only expressed in large amounts during the very late stages of infection, and it is still unclear whether cell death is due to apoptosis. Although E1B-19K protects infected cells from apoptosis, at late stages of infection its effect is eventually overcome, allowing cell death to proceed and viral progeny to spread to neighboring cells. Although no direct evidence yet exists, an intriguing possibility is that one function of the ADP is to block the inhibitory effects of 19K.

Alphavirus. The cytopathic effect of Sindbis virus infection is, at least in part, mediated by apoptosis (46). Early studies which examined the ultrastructural features of Sindbis virus-infected cells clearly showed the presence of extensive vacuolization and condensed chromatin which are considered hallmarks of apoptosis (30). Sindbis virus is a clear example of the central role of apoptosis in the infectious process, as overexpression of Bcl-2 dramatically shifts the infectious cycle from a lytic to a persistent phase (46). The induction of apoptosis in Sindbis virus-infected cells has also been demonstrated to contribute to pathogenicity in vivo. Sindbis virus-infected neurons of the central nervous system (CNS) of mice were observed to die by apoptosis, and thus this process may represent the mechanism of neurovirulence of the virus (47).

Lentiviruses. One clinically significant example of virus-induced apoptosis relates to the replication of human immunodeficiency virus type 1 (HIV-1). Patients infected with HIV-1 characteristically undergo selective depletion of $CD4^+$ T cells, eventually resulting in the incapacitation of the immune system. T cells from HIV patients have been demonstrated to be sensitive to activation-induced apoptosis (34). Recent studies have shown that the underlying process which triggers the T-cell depletion is the induction of apoptosis in HIV-infected cells (48). The molecular mechanism of HIV-1-induced apoptosis involves Fas, a receptor on the surface of lymphoid cells which induces apoptosis upon binding of Fas ligand. The viral transcription factor, Tat, is able to up-regulate expression of Fas ligand and thus induce apoptosis (94). Overexpression of Fas ligand also leads to the death of many uninfected T cells in the proximity of infected cells (27). Such virus-induced apoptosis may play a significant role in the pathogenesis of the AIDS virus. Tat expression has also been shown to downregulate the expression of Bcl-2 while causing increased expression of the apoptosis accelerator Bax (71). Another human lentivirus, human T-cell leukemia virus, which encodes a Tat homolog, Tax, may also induce apoptosis via similar mechanisms (99). In addition to the loss of $CD4^+$ T lymphocytes, HIV-infected individuals often develop dementia which is associated with the loss of neurons in discrete regions of the CNS. Using simian immunodeficiency virus (SIV)-infected macaques as a model, one group has reported that this loss of neurons is mediated by apoptosis most likely caused by SIV-infected lymphoid cells which infiltrate into CNS tissue (1).

Parvoviruses. Another virus of clinical importance, the B19 human parvovirus, induces morphological changes in infected cells which resemble those associated with apoptosis (57). Toxicity of B19 virus seems to originate from a single viral gene which encodes the nonstructural protein NSP. NSP proteins from both human B19 and murine parvoviruses were shown to be lethal when expressed alone in various cell lines (9, 62). The mechanism by which NSP induces cell death is not known, but the protein appears to be localized in the nucleus and has DNA binding properties (19).

Arteriviridae. Porcine reproductive and respiratory syndrome virus (PRRS) is a member of a proposed new family of viruses called the *Arteriviridae*. PRRS has been demonstrated to induce apoptosis in the course of infection, and this activity has been directly mapped to open reading frame (ORF) 5 (p25) of the virus (80). The p25 protein is believed to be a membrane-associated glycoprotein which is present in virus particles (55). The mechanism by which apoptosis is induced by p25 is not known, but a very interesting aspect of p25-mediated cell death is that it cannot be inhibited by Bcl-2, suggesting either the existence of a target which is downstream of Bcl-2 family members (e.g., ICE proteases) or an alternative pathway of apoptosis (80).

Other viruses. Cells infected by a growing number of other viruses have been reported to display properties typical of apoptosis. Such viruses include measles virus, influenza virus, various herpesviruses, infectious bursal disease virus, bovine diarrhea virus, Dengue virus, vesicular stomatitis virus, Newcastle disease virus, and La Crosse virus. In most of these cases it is still unclear which viral products are involved. Nevertheless, it is obvious that a great deal of information will be forthcoming on the role of apoptosis in the life cycles of a great number of viruses.

SPECULATIONS

As described above, there is increasing evidence that many animal viruses encode proteins which interact intimately with the biochemical pathways regulating apoptosis. Viruses which induce cell cycle progression in the course of infection have evolved disparate methods of evading the toxic effects of p53 which is activated after infection. With some viruses, inhibition of apoptosis seems to be essential for the maintenance of viral latency. And for many viruses, the carefully choreographed induction of apoptosis during lytic infection may represent the basis for cytotoxicity and an important outlet for dissemination of progeny virus. As these processes are understood in more detail, opportunities for the development of new drugs to combat clinically important viruses will almost certainly arise. Such drugs could promote the early death of infected cells, inhibit virus release, or in the case of latent viruses, manipulate the lytic-latency switch to minimize the effects of infection. These treatments may also prove to be less susceptible to mutational

adaptation by viruses as is the case with many drugs which function by inhibiting viral enzymes.

Clearly, as details of the infectious processes of more viruses become known, tremendous insights into the molecular biology of apoptosis will be forthcoming. One important area concerns the Fas and TNF pathways. Further understanding of the functions of inhibitory viral proteins should be very informative. Ad E3 proteins appear to block the early stages of signaling and may mimic natural cellular controls. IAP proteins are clearly related to a family of important cellular regulators which function by a poorly understood mechanism via the RING and BIR domains. As was the case with Rb-binding proteins and BH-containing polypeptides, it is likely that identification and characterization of functional domains within IAP species will be highly informative. Finally, as Ad E1B-19K blocks the Fas and TNF pathways, 19K may be used to establish new links between the Bcl-2 and ICE protease setpoints.

Although information is accumulating rapidly about the mechanism of induction of apoptosis by TNF, Fas ligand, and other death domain proteins, including the *Drosophila* Reaper polypeptide (28), much remains to be learned about these and other inducers of cell death. Many viruses induce p53-dependent or p53-independent apoptosis, and the viral functions responsible are currently being identified and characterized. It is likely that the identification of viral killing domains may lead to a better understanding of Bax, TNF, and other as yet unknown cellular polypeptide inducers of apoptosis.

Finally, can information about viruses and apoptosis be used to develop new therapies for human diseases? The answer is almost certainly yes. A great many human diseases seem to result from the inappropriate occurrence of apoptosis (neurodegenerative diseases and cytolytic maladies such as AIDS) or the failure to initiate cell death pathways (autoimmune diseases and cancer). It may be possible to adapt viral suppressors of apoptosis to block untimely cell death and prolong the lifetime of affected cells. In the case of cancer, the failure of cells to die in response to the expression of activated oncogenes and other stimuli results in the persistence and accumulation of neoplastic cells. One frequent mechanism that cancer cells employ to evade apoptosis is the inactivation of p53. Many current cancer treatments actually work by inducing p53-dependent apoptosis, and thus such p53 null tumor cells are particularly resistant to killing. As discussed above, several viruses kill cells through the activation of p53-independent apoptosis. Thus, such viruses could be used to kill p53-deficient cancer cells. Interestingly, just such a strategy is being tested using an Ad mutant with a defect in the E1B-55K protein (4). This mutant was found to replicate preferentially in p53 null cells, as p53 was found to block viral replication in the absence of 55K. As described above, Ad induces p53-independent cell death (53, 82, 86). Thus, injection of this mutant Ad into p53 null tumors could preferentially kill the cancer cells while having little effect on normal tissues. Viral death inducers could also be adapted to kill such cancer cells directly through a process that does not rely on viral replication. Thus, continued investment in virus research may once again prove to be highly justified.

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