Control of Apoptosis by Human Cytomegalovirus

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Contents

Abstract Caspase-dependent apoptosis has an important role in controlling viruses, and as a result, viruses often encode proteins that target this pathway. Caspase-dependent apoptosis can be activated from within the infected cell as an intrinsic response to replication-associated stresses or through death-inducing signals produced extrinsically by immune cells. Cytomegaloviruses (CMVs) encode a mitochondria-localized inhibitor of apoptosis, vMIA, and a viral inhibitor of caspase activation, vICA, the functional homologs of Bcl-2 related and c-FLIP proteins, respectively. Evidence from viral mutants deleting either vMIA or vICA suggests that each is necessary and sufficient to promote survival of infected cells undergoing caspase-dependent apoptosis. Additional proteins, including pUL38, $IE1_{491aa}$, and $IE2_{579aa}$, can prevent apoptosis induced by various stimuli, while viruses with deletions of UL38, M45, or m41 undergo apoptosis. The viral RNA, β2.7, binds mitochondrial respiratory complex I, maintains ATP production late in infection, and prevents death induced by a mitochondrial poison. Thus, CMV

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alters cell intrinsic defenses employing apoptosis, and multiple viral gene products together control death-inducing stimuli to promote survival.

Introduction

Apoptosis is an evolutionarily conserved process that removes cells during development and homeostasis and that can limit viral replication (Roulston et al. 1999). Apoptosis results from the hierarchical activation of a family of cysteine proteases, the caspases, that follows extrinsic or intrinsic pro-death signaling (Festjens et al. 2006). Extrinsic signals engage death receptors, a subset of the TNF superfamily, promoting the recruitment of cytoplasmic proteins and activation of initiator caspase-8. This caspase is highly regulated, including by c-FLIP proteins that can prevent proteolytic activation (Barnhart et al. 2003). Intrinsic signals following DNA damage, ER stress, or other stresses alter mitochondria membrane permeability, promote release of cytochrome c and additional pro-death factors, and activate initiator caspase-9 (Festjens et al. 2006). Both extrinsic and intrinsic pathways converge on downstream executioner caspase-3, which targets specific proteins. For most types of cells, extrinsic signals are also amplified by way of mitochondrial alterations and caspase-9 activation (Barnhart et al. 2003). The cellular Bcl-2 family proteins tightly regulate the mitochondria membrane permeability transition (Kuwana and Newmeyer 2003; Green and Kroemer 2004; Sharpe et al. 2004; Antignani and Youle 2006). Proteins in this family include one of four distinct amino acid sequence domains, known as Bcl-2 homology (BH) domains that are important to function (Petros et al. 2004). The balance of pro- and antiapoptotic Bcl-2 proteins determines whether a cell undergoes apoptosis (Kuwana and Newmeyer 2003; Green and Kroemer 2004; Sharpe et al. 2004; Antignani and Youle 2006). The proapoptotic proteins Bax and Bak are directly linked to the release of pro-death factors from mitochondria. While still controversial, one suggested mechanism employs the inherent pore-forming properties of these proteins. The actions of the proapoptotic Bcl-2-related proteins are balanced by antiapoptotic Bcl-2 and Bcl-x_L. Likewise, pro-death signals can be balanced by more global pro-survival signals. Sensors located in various organelles, including the nucleus, endoplasmic reticulum, lysosomes, and the Golgi apparatus can promote death through apoptosis (Ferri and Kroemer 2001); thus, events leading to death can occur from multiple cellular sites. Many viral factors that counteract caspase-dependent apoptosis are homologs of key cellular regulatory proteins, including the Bcl-2 related proteins and the c-FLIP proteins (Irusta et al. 2003; Polster et al. 2004).

CMV genes that impact apoptosis have been identified by three different strategies. In the first, the antiapoptotic designation followed transient expression and increased cell survival in well-defined models of apoptosis. A random search employing this strategy uncovered the viral mitochondria-localized inhibitor of apoptosis (vMIA) encoded by UL37×1 and the viral inhibitor of caspase-8 activation (vICA),

encoded by UL36 (Goldmacher et al. 1999; Skaletskaya et al. 2001). Both functions increase infected cell resistance to apoptosis (Skaletskaya et al. 2001; Menard et al. 2003; Reboredo et al. 2004; McCormick et al. 2005). A direct assessment of $IE1_{491aa}$ and $IE2_{579aa}$ lead to observed impacts on pro-survival signaling mediated by the kinase Akt (Lukac and Alwine; Yu and Alwine 2002). The mechanisms and direct impact of these pro-survival activities on viral growth remain unexplored. Viral genetics highlighted the contributions of UL38, M45, and m41 to survival from apoptosis induced by replication (Brune et al. 2001, 2003; Terhune et al. 2007). Although the antiapoptotic mechanism remains unknown, pUL38 is sufficient to increase survival in apoptosis models (Terhune et al. 2007). Lastly, interaction studies revealed the RNA, β2.7, binds mitochondrial complex I and as a result, controls mitochondrial function and cell survival following death induced by respiration poisons. Thus, multiple CMV genes encode pro-survival or antiapoptotic factors. The phenotypes of viral mutants combined with results of exogenous expression analyses, suggest the UL36-38 genomic region is a cell death suppression locus.

vMIA Controls Mitochondria-Dependent Death

The UL37×1 ORF encoding vMIA is included on multiple viral transcripts. (Tenney and Colberg-Poley 1990, 1991a, 1991b; Goldmacher et al. 1999) (Fig. 1). The predominant, unspliced transcript yields the 163 aa vMIA, while splicing to UL37×2 and UL37×3 yields the larger antiapoptotic glycoprotein gpUL37 and $pUL37_M$ (Goldmacher et al. 1999). Additional less abundant spliced transcripts are predicted to encode antiapoptotic proteins as well, but have not yet been tested for function (Adair et al. 2003). vMIA localizes to mitochondria and prevents the release of pro-death

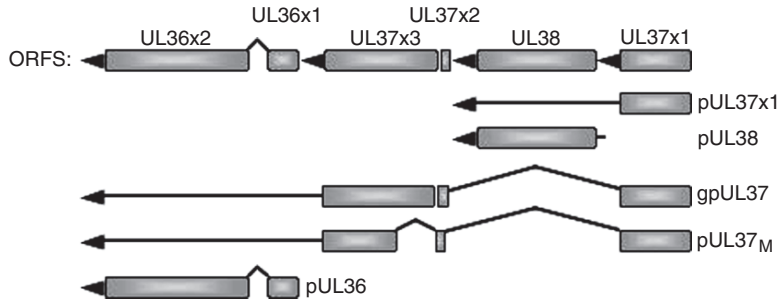

Fig. 1 A map of the HCMV UL36-UL38 cell death suppression locus indicating the relative positions of open reading frames (ORFs) and major transcripts of the region. *Rectangles* represent the ORFs and include an *arrowhead* to denote the direction of transcription. *Arrows* represent the 3′ nontranslated regions. A *raised line* connecting ORFs indicates splicing. Splicing events producing minor transcripts of the UL37 gene (Adair et al. 2003) are not shown

factors similar to Bcl-2 or Bcl-x_L (Goldmacher et al. 1999). To date, vMIA is the most broadly antiapoptotic CMV protein known and analogous to the cellular Bcl-2 proteins, is highly effective against a myriad of stimuli including intrinsic stresses as well as extrinsic, immune-regulated signals (Goldmacher et al. 1999; Belzacq et al. 2001; Vieira et al. 2001; Jan et al. 2002; Roumier et al. 2002; Boya et al. 2003; Andreau et al. 2004; Arnoult et al. 2004; Boya et al. 2005; McCormick et al. 2005). However, vMIA does not encode any BH-domains that characterize the cellular proteins (Goldmacher et al. 1999).

vMIA function requires an amino terminal mitochondrial-targeting domain (aa 2-34) and a carboxyl-terminal antiapoptotic domain (AAD, aa 118-147) (Hayajneh et al. 2001) that together are sufficient for function. The mitochondrial-targeting domain includes an amino-terminal hydrophobic signal followed by highly conserved basic residues, and both are required for mitochondrial trafficking (Mavinakere and Colberg-Poley 2004). Evidence suggests a mitochondrial membrane association with the targeting domain spanning the membrane and the AAD exposed to the cytoplasm (Mavinakere et al. 2006). The carboxyl-terminal AAD includes a predicted amphipathic α-helix motif (aa 126-140) critical to function (Smith and Mocarski 2005). Point mutations predicted to disrupt an α-helical structure alter amphipathicity or place charge on the hydrophobic face of the AAD α-helix, each completely abrogate vMIA function. In contrast, the hydrophilic face of the AAD α-helix tolerates significant substitutions with as many as five or six amino acid substitutions required to disrupt function (Smith and Mocarski 2005).

The growth arrest and DNA damage 45 alpha (GADD45α) protein interacts directly with vMIA in yeast and mammalian cells, fails to bind vMIA mutant proteins, and is essential for vMIA-mediated antiapoptotic activity (Smith and Mocarski 2005). Targeted knockdown of GADD45α, GADD45β, and GADD45γ reduced vMIA activity, and each GADD45 family protein individually enhanced vMIA activity. GADD45α increased both the overall amount of vMIA and that associated with mitochondrial fractions. Thus, the DNA damage response pathway is directly linked to vMIA-mediated cell death suppression. Further, vMIA was shown to bind the antiapoptotic Bcl-2 family protein Bcl-x_L in mammalian cells. Collectively, these data suggest that vMIA acts together with Bcl-x_L and GADD45 to regulate the mitochondrial release of proapoptotic factors (Fig. 2).

In addition to GADD45 proteins, vMIA also binds the proapoptotic Bcl-2 family protein Bax (Arnoult et al. 2004), which has more recently been connected to mitochondrial morphogenesis during life (Karbowski et al. 2006). In most instances, Bax is distributed in the cytoplasm, but Bax oligomerization and relocalization to mitochondria mediates the release of proapoptotic factors from the organelle (Antonsson et al. 2001). In the presence of vMIA, however, oligomerized Bax at mitochondria fails to promote apoptosis, suggesting sequestration as a component of the antiapoptotic mechanism (Arnoult et al. 2004; Poncet et al. 2004). Thus, the vMIA-dependent antiapoptotic mechanism is distinct from that of cellular and viral Bcl-2 proteins that prevent Bax relocalization and oligomerization at mitochondria. Recruitment and sequestration

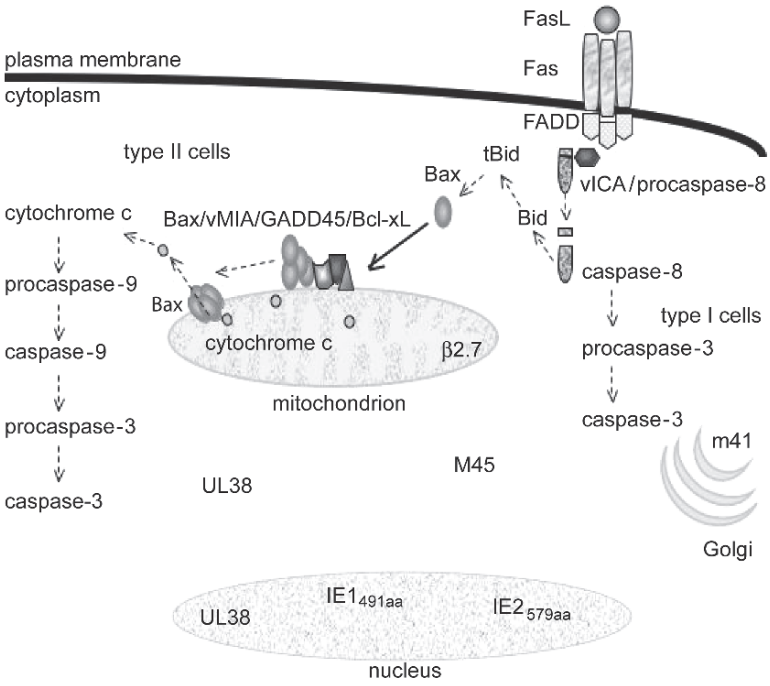

Fig. 2 A representation of the apoptosis pathway and CMV-mediated alterations preventing death, as described in the text. *Dashed arrows* indicate events prevented by the viral proteins, vICA or vMIA, as indicated. The *solid arrow* indicates vMIA-dependent relocalization of Bax to mitochondria. At mitochondria, a complex(s) of proteins including Bax as an oligomer, vMIA, GADD45, and Bcl-x_L prevents the release of mitochondrial protein cytochrome c. vICA binds procaspase-8 and is depicted as a complex that prevents procaspase-8 activation following addition of extrinsic death signals. The mechanisms and/or direct physical interactions that promote survival in the presence of the remaining viral proteins, pUL38, $IE1_{491aa}$, $IE2_{579aa}$, M45, and m41 are unclear, and these are placed according to the anticipated site of localization within the cell. For simplicity, many important regulatory components have not been included

of Bax at mitochondria has also been suggested as the mechanism (Karbowski et al. 2006) for vMIA-dependent disruption of reticular mitochondrial networks (McCormick et al. 2003b); however, more recent evidence of vMIA mutants that disrupt networks but fail to bind Bax (Pauleau et al. 2007) suggests other factors may also be important.

vMIA prevents apoptosis during infection; however, vMIA is not required for replication, and replication in the absence of vMIA does not induce caspase-dependent apoptosis (McCormick et al. 2005). A vMIA deletion mutant made in the laboratory-propagated strain, Towne*var*ATCC, produces yields nearly equivalent to parental virus. In contrast, vMIA is more critical for efficient replication of the laboratory strain AD169*var*ATCC (Reboredo et al. 2004). These strain-dependent variations may suggest that the quantity or quality of intrinsic stresses

produced by individual strains varies because evidence suggests vMIA is an important regulator of the viral response to stress (McCormick et al. 2005). The phenotype produced by disruption of vMIA in AD169*var*ATCC is highly variable (Brune et al. 2003; Yu et al. 2003; Sharon-Friling et al. 2006), perhaps due to other factors that prevent vMIA-dependent release of calcium from the endoplasmic reticulum (Sharon-Friling et al. 2006) or increase ATP levels (Poncet et al. 2006), and further analyses are needed to resolve the role of vMIA in that strain.

Although of limited impact on replication in HFs, the Towne*var*ATCC mutant revealed a role for vMIA in regulating caspase-independent death. Caspase-3 activation underlies caspase-dependent apoptosis; however, this protease is not required for other cell death pathways that are considered to be apoptosis-like (Leist and Jaattela 2001; Lockshin and Zakeri 2002; Jaattela 2004). Thus, UL37×1 deletion can promote CMV-induced caspase-3-dependent cell death in the case of AD169*var*ATCC (Reboredo et al. 2004), or a caspase-3-independent cell death in the case of Towne*var*ATCC (McCormick et al. 2005), and vMIA regulates both forms of death during infection (McCormick et al. 2005). From studies so far, the context where caspase-3-independent cell death is a significant obstacle to the virus is unknown.

Chimpanzee CMV, rhesus macaque CMV, and African green monkey CMV each retain a vMIA homolog that could be identified through computer analysis (McCormick et al. 2003a). Each of these proteins share sequence similarity with the mitochondrial-targeting and AAD domains of vMIA. Rhesus macaque CMV vMIA retains similarity only to the amino- and carboxyl-terminal domains of human CMV (HCMV) vMIA and functions as an antiapoptotic protein. It is expected that all primate CMVs encode functional homologs. In contrast, the identification of rodent CMV functional homologs encoded by ORFs, m38.5 and r38.5, required more extensive analyses due to limited sequence homology (McCormick et al. 2003a, 2005; Brocchieri et al. 2005). Initial searches for murine CMV (MCMV) mitochondrial localized proteins with vMIA function were executed in HeLa cells utilizing methods that revealed vMIA (Goldmacher et al. 1999; McCormick et al. 2003a). Increasing the repertoire of stimuli revealed that m38.5 prevents proteasome inhibitor-induced, intrinsic apoptosis but not extrinsic, Fas-mediated apoptosis in HeLa cells (McCormick et al. 2005) or a telomerase-immortalized retinal epithelial cell line of human origin (Jurak and Brune 2006). Thus, MCMV m38.5 encodes an antiapoptotic protein that localizes to mitochondria (McCormick et al. 2005). The rodent CMV ORFs map to positions on the viral genomes analogous to UL37×1 (McCormick et al. 2003a; Brocchieri et al. 2005), indicating that rodent and primate CMVs each encode vMIA and vICA homologs.

Limited sequence similarity and differences in protective function in human cells suggest the human and rodent vMIA homologs retain elements that are specific to function in the appropriate host (McCormick et al. 2005). Identification of additional MCMV proteins localized to mitochondria may also suggest the potential for synergism or even replacement of m38.5 function in specific cells (Tang et al. 2006). Interestingly, vMIA apparently protects from specific apoptotic

stimuli in a species-dependent fashion as well. Thus, vMIA fails to prevent mitochondrial damage induced by staurosporine in wild type murine fibroblasts, apparently due to the role of murine Bak in that setting (Arnoult et al. 2004) but prevents staurosporine-induced death in HeLa cells (Andreau et al. 2004). Thus, properties of the antiapoptotic proteins encoded by these viruses reflect the evolutionary divergence of the host. In fact, one aspect of the species barrier that restricts CMVs is reportedly due to functions that for MCMV can be provided by vMIA (Jurak and Brune 2006). Given the genomic organization and studies thus far, it is likely that m38.5 will retain vMIA functions relevant to survival in the host and that all CMVs rely on vMIA function.

vICA Controls Caspase-8

vICA, the UL36 gene product, interferes with caspase-8-dependent apoptosis by binding procaspase-8 and preventing proteolytic activation (Skaletskaya et al. 2001) (Fig. 2). The role of caspase-8 as an initiator protease activated by extrinsic, immune-regulated signals implies vICA is important to survival in the host (Skaletskaya et al. 2001). vICA is highly conserved among mammalian betaherpesviruses both in sequence and function, suggesting a conserved biologic role (McCormick et al. 2003a; Menard et al. 2003). In contrast, passage in tissue culture has promoted adventitious mutations that impact antiapoptotic function (Skaletskaya et al. 2001). Although early work employed a recombinant virus made in AD169*var*ATCC, a laboratory strain that had already acquired mutations in vICA (Patterson and Shenk 1999; Skaletskaya et al. 2001), deletion of the gene from Towne-BAC, a viral strain that retains vICA function, confirmed that both the UL36 gene and vICA function can be altered without impacting replication in cultured fibroblasts (Dunn et al. 2003). MCMV mutants impacting M36 also grow in fibroblasts; however, this gene is required for growth in cultured macrophages (Menard et al. 2003) and in mice (Cicin-Sain et al. 2005). Importantly, infected macrophages elevated caspase-8 activity only in the absence of M36 (Menard et al. 2003). These observations are consistent with the expectation that vICA prevents caspase-8 activation, thereby performing a critical role for survival in the host.

$IE1_{491aa}$, $IE2_{579aa}$, and Akt-Dependent Pro-survival Pathways

Pathways leading to death are balanced by pro-survival pathways, including those regulated by trophic factors that signal through phosphatidylinositide 3′-OH kinase (PI3K)/Akt kinase (Datta et al. 1999). Evidence suggests CMV requires the PI3K/Akt pathway for replication (Johnson et al. 2001). The $IE1_{491aa}$ and $IE2_{579aa}$ are nuclear proteins that regulate transcription and have important roles in viral replication (see the chapter by M.F. Stinski and D.T. Petrik, this volume and Stinski and

Meier 2007; White and Spector 2007). Each has been connected to the PI3K/Akt pro-survival pathway through the following. Initially, antiapoptotic roles for $IE1_{491aa}$ and $IE2_{579aa}$ were suggested from results of transient and stable expression in HeLa cells (Zhu et al. 1995). Here, either protein protects from short exposure (8 h) to TNF or infection by E1B-19-kDa-deficient adenovirus, but not from UV irradiation. Mechanisms are suggested to differ because antiapoptotic activity maps to unique sequences. In comparison with vMIA, neither $IE1_{491aa}$ nor $IE2_{579aa}$ protect HeLa cells undergoing TNF- or Fas-mediated apoptosis when evaluated in a more rigorous assay (24 h) (Goldmacher et al. 1999). Thus, these proteins present challenges for mechanistic studies.

One experimental approach that has suggested the mechanism of $IE1_{491aa}$ and $IE2_{579aa}$ antiapoptotic function employed the temperature-sensitive (*ts*) BHK-21 cell line *ts*13 (Lukac et al. 1997; Lukac and Alwine 1999; Yu and Alwine 2002). At the nonpermissive temperature, a mutation in $TAF_{II}250$ produces transcription alterations in specific genes that results in a block to cell cycle progression and the induction of apoptosis in these hamster cells (Talavera and Basilico 1977; Sekiguchi et al. 1988, 1995). When expressed from a genomic construct, $IE1_{491aa}$ and $IE2_{579aa}$ prevent apoptosis and rescue promoter-specific transcription through independent mechanisms that do not rescue cell cycle defects (Lukac et al. 1997; Lukac and Alwine 1999). Further, IE rescue of transcription is primarily due to $IE2_{579aa}$ (Lukac et al. 1997), while either IE protein rescues apoptosis (Yu and Alwine 2002). Protein domains required for protective function remain unidentified. Although the protective mechanism in this setting relies on PI3K activation of Akt, how $IE1_{491aa}$ and $IE2_{579aa}$ promote this activation is unknown, as is the significance of these results to infection. Nevertheless, these studies suggest hypotheses that may elucidate $IE1_{491aa}$ and $IE2_{579aa}$ contributions to survival of infected cells.

UL38 Decreases Intrinsic Stress

The UL38 ORF maps to an intron of the UL37 gene (Tenney and Colberg-Poley 1990, 1991a, 1991b) (Fig. 1). The UL38 sequence is included on the unspliced vMIA transcript and on a unique transcript with early kinetics. During infection, pUL38 initially localizes to the nucleus but is well distributed between the nucleus and cytoplasm by 24 h (Terhune et al. 2007). Mutagenesis of the UL38 ORF in either Towne-BAC (Dunn et al. 2003) or pAD/Cre (Yu et al. 2002; Terhune et al. 2007) reduces yield by approximately 100-fold during a single round of replication (Terhune et al. 2007).

The premature death induced by the UL38-null mutant virus and rescue by addition of the pan-caspase inhibitor zVAD-fmk prompted further analysis of pUL38 as an antiapoptotic factor (Terhune et al. 2007). Replication of the UL38 deletion mutant is also largely restored by zVAD-fmk. Death initiates very early (24 h) and reaches more than 50% by 72 h, suggesting pUL38 is required very

early. Consistently, TUNEL labeling, expected to reflect caspase-3-mediated activation of nucleases, occurs by 48 h. In contrast, detection of active caspase-3 or cleaved substrate (PARP) is apparently variable and occurs consistently only by 96 h. Thus, evaluation of specific steps along the apoptosis pathway may highlight important viral controls. Nevertheless, a deletion mutant of UL38 in pAD/Cre induces an apoptotic death that is repaired by growth on pUL38-expressing cells. pUL38 is sufficient to inhibit death induced by E1B-19-kDA-deficient adenovirus or thapsigargin, but is ineffective against Fas-mediated apoptosis. Thus, pUL38 protects from intrinsic but not extrinsic death signals. Thus far, little is known of the pUL38-dependent antiapoptotic mechanism or protein domains required for function, but these studies will likely be included in future endeavors.

M45 Is a Cell Type-Specific Survival Factor

Betaherpesviruses, including HCMV, encode the UL45 genes that are related by sequence but not function to ribonucleotide reductase (Chee et al. 1990; Patrone et al. 2003; Lembo et al. 2004). Viral mutants that disrupt the MCMV M45 gene induce apoptosis and are growth-restricted in endothelial cells and macrophages but not fibroblasts, bone marrow stromal cells, or hepatocytes (Brune et al. 2001). Although early and late genes are expressed, infection induces apoptosis and infectious progeny are not produced. Assays predictive of apoptosis, including nuclease activity and phosphatidylserine exposure, implicate this pathway; however, a direct link between decreased apoptosis and rescued growth has not been established. Further, M45-dependent survival in apoptosis models or viral replication has not been demonstrated, and it is unclear how the phenotype of the mutant virus relates to apoptosis pathways and M45. However, disruption of M45 produces a nonpathogenic virus (Lembo et al. 2004), and further studies will likely answer these important questions.

Neither replication in endothelial cells or resistance to induced intrinsic stress requires HCMV UL45 (Hahn et al. 2002). Thus, the intrinsic stresses revealed by deletion of MCMV M45 are apparently controlled by other viral factors in HCMV. In contrast, UL45 does increase viral production of the laboratory strain AD169*var*ATCC in fibroblasts following a low multiplicity infection (Patrone et al. 2003). However, decreased yield does not result from increased apoptosis. The resistance of HCMV to extrinsic but not intrinsic apoptosis is also halved, but the contribution of UL45 is unknown and UL45-expressing fibroblasts remain sensitive to Fas-mediated apoptosis. Considerable effort has been required to evaluate the potential of UL45 as a ribonucleotide reductase (Patrone et al. 2003; Lembo et al. 2004). Future studies that define the UL45 function that increases low multiplicity growth and the M45 function that permits replication in endothelial cells will likely clarify the role of this perplexing gene.

m41, Late Infection, and the Golgi Apparatus

The CMV ORFs m41 and r41 are apparently unique to rodent CMVs and, as such, do not share sequence homology with HCMV ORFs (Chee et al. 1990; Rawlinson et al. 1996; Mocarski et al. 1997; Vink et al. 2000; Brocchieri et al. 2005). Expression constructs produce a single protein of 19 kDa while polypeptides of 19 and 21-kDa polypeptides are produced during infection, suggesting splicing to upstream or downstream ORFs (Brune et al. 2003). Recombinants that disrupt the m41 ORF induce apoptosis very late in infection, as suggested both by apoptosis-induced molecular changes, including phosphatidylserine exposure and nuclease-driven chromatin alterations, and increased survival in the presence of caspase inhibitors. A more dramatic impact on replication occurs in endothelial cells where viral yields are reduced 50-fold. Thus far, neither yield nor death has been directly related to pm41, but Golgi localization is likely important to pm41 function.

β2.7 and Mitochondrial Respiratory Complex I

The highly abundant early transcript β2.7 (McDonough and Spector 1983; McDonough et al. 1985) is polysome-associated (Wathen and Stinski 1982); however, sequence analyses suggest a noncoding RNA (McSharry et al. 2003). Although the gene is conserved in both laboratory-adapted viral strains and clinical isolates, the RL4 ORF is not. Northwestern screening suggested β2.7 binds proteins of the nicotinamide adenine dinucleotide-ubiquinone oxidoreductase (mitochondrial respiration complex I) (Reeves et al. 2007). β2.7 also increases survival from the mitochondrial poison rotenone and maintains ATP production late in infection. The importance of continued mitochondrial function during CMV infection has been suggested from several studies that have evaluated mitochondrial DNA synthesis, mitochondrial protein expression profiles, and ATP production (Furukawa et al. 1976; Hertel and Mocarski 2004; Reeves et al. 2007). β2.7 contributes to viral production by maintaining ATP production (Reeves et al. 2007), and in addition, prevents death that follows intrinsic stresses associated with decreased ATP.

Summary and Perspectives

Several CMV gene products that impact apoptosis have already been identified. vMIA and vICA are the most extensively characterized with regard to proposed mechanism and antiapoptotic roles during infection. pUL38, M45, and m41 remain largely uncharacterized with regard to mechanism, and the contributions of $IE1_{491aa}$ and $IE2_{579aa}$ antiapoptotic functions during replication remain to be addressed. Additional genes like β2.7, which increase survival in response to stress, are likely to be identified through efforts that determine viral control of cellular stress responses (see the chapter

by A.L. Alwine, this volume) and these genes will also impact viral control of apoptosis. Three types of cell death-apoptosis, necrosis, and autophagy-have been well characterized by morphology, biochemical events, and host responses (Leist and Jaattela 2001; Jaattela 2004; Lockshin and Zakeri 2004; Vandenabeele et al. 2006; Golstein and Kroemer 2007). Given overlapping regulation and expectations from cellular homologs with cross-inhibitory properties, future efforts will undoubtedly reveal as yet unappreciated connections between CMV antiapoptotic proteins and other cell death pathways. In summary, the cell tropism of CMV (Mocarski et al. 2006) likely means the virus must be armed against multiple forms of death and the combination of all suppressors encoded by the virus likely balances the apoptotic threshold in a direction supporting replication.

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