
Cell Death Suppressors Encoded by Cytomegalovirus

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1

Introduction

Apoptosis of virally infected cells is a host defense mechanism designed to arrest viral replication by eliminating infected cells (O'Brien 1998). Apoptosis induced by virus infection can be triggered intrinsically by initiating an apoptotic pathway in response to the metabolic changes caused by the infection. The cytotoxic effector cells of the immune system can induce apoptosis in virally infected target cells via multiple mechanisms such as ligation of cell surface death receptors (Wallach et al. 1999), or the release of proteases (granzymes) on contact with target cells (Smyth et al. 2001). A number of viruses evolved with elaborate, often several, strategies to prevent or delay apoptotic host responses (O'Brien 1998; Tschopp et al. 1998). In this article I will review the current status of our knowledge on the cell-death suppressing strategies employed by cytomegaloviruses, a subset of β -herpesviruses.

Until recently, virtually nothing was known about the mechanisms of cell-death suppression by β -herpesviruses. There were reports that productive infection with human cytomegalovirus (CMV) rendered cells resistant to apoptosis induced by diverse pro-apoptotic stimuli [reviewed in Goldmacher et al. (1999)], but the nature of these phenomena remained obscure. Homology searches of the genome of human CMV failed to reveal any genes homologous to any known cell-death suppressor.

A functional screen of a CMV genomic library (Goldmacher et al. 1999; Skaletskaya et al. 2001) enabled us to identify two CMV-encoded cell-death suppressors, vICA (viral inhibitor of caspase-8-induced apoptosis) and vMIA (viral mitochondria-localized inhibitor of apoptosis). vICA and vMIA have functional similarities with c-FLIP or Bcl-2, respectively, but share no sequence homology with these proteins. These cell-death suppressors are rather unique: to date, no cellular or viral homologues of vICA or vMIA have been identified except those encoded by other β -herpesviruses (McCormick et al. 2003).

In addition to vICA and vMIA, several other CMV-encoded proteins, IE1 and IE2 of human CMV, and pM45 of mouse CMV, were reported to be capable

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of protecting cells from apoptosis. However, the evidence that these proteins are bona fide cell-death suppressors, and are involved in protection of CMV-infected cells from apoptosis, is inconclusive (see below).

2

vICA, a Cell-Death Suppressor That Blocks Caspase-8 Activation

2.1

Cell-Death Suppressing Activity of vICA

vICA, also known as pUL36, is encoded by the *UL36* gene of human CMV. The anti-apoptotic activity of vICA was demonstrated in stably transfected BJAB and HeLa cells, which are type I and type II cells, respectively (Skaletskaya et al. 2001). During death-receptor ligation-mediated apoptosis in type I cells, activation of caspase-8 leads directly to activation of caspase-3 bypassing the mitochondrial apoptotic signaling pathway, while in type II cells activation of caspase-3 can only be achieved through the mitochondrial apoptotic pathway (Scaffidi et al. 1998, 1999b; Schmitz et al. 1999). Ectopic expression of vICA protected both cell lines against Fas-mediated apoptosis. In this respect, vICA is similar to cFLIP, a cell-death suppressor that blocks caspase-8 activation. cFLIP is active both in type I (Scaffidi et al. 1999a) and type II cells (Scaffidi et al. 1999b; Engels et al. 2000). vICA protects cells against apoptosis triggered by ligands of other death receptors (tumor necrosis factor receptor I and Apo-2), but appears to only marginally protect cells against apoptosis induced by either the cytotoxic drugs doxorubicin or maytansine, or by infection with an E1B19k-deficient adenovirus.

2.2

vICA Prevents Caspase-8 Activation and Forms a Complex with Pro-Caspase-8

Ligation of cell-surface Fas triggers the recruitment of pro-caspase-8 to the death-inducing signaling complex (DISC) through the death domain-containing adapter protein FADD, and sets off activation of pro-caspase-8 by means of proteolytic processing (Krueger et al. 2001). Biochemical data indicate that vICA interrupts the Fas-ligation-mediated apoptotic pathway at, or upstream of, caspase-8 activation: vICA inhibits Fas-ligation-mediated activation of caspase-8 and downstream events of the apoptotic signaling pathway: proteolytic processing of BID, a substrate of caspase-8, and proteolytic processing of PARP, a substrate of downstream caspases (Skaletskaya et al. 2001). vICA has affinity to pro-caspase-8, specifically to its pro-domain region that contains

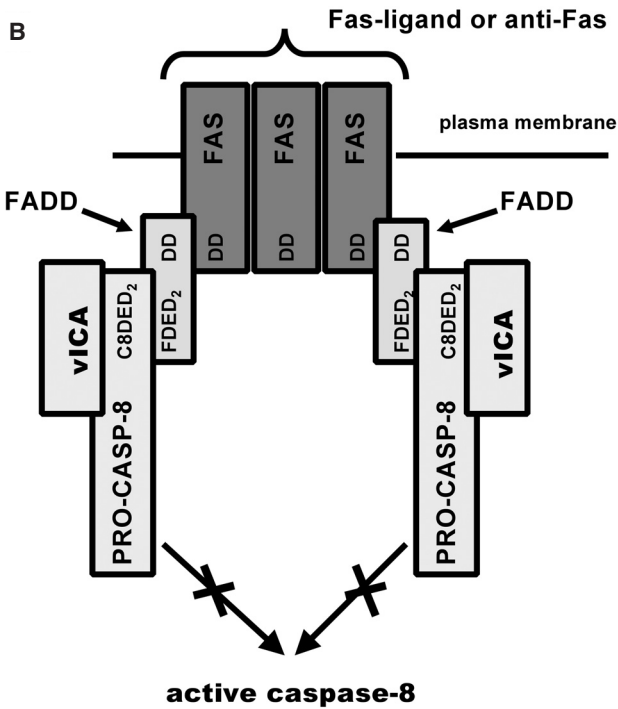
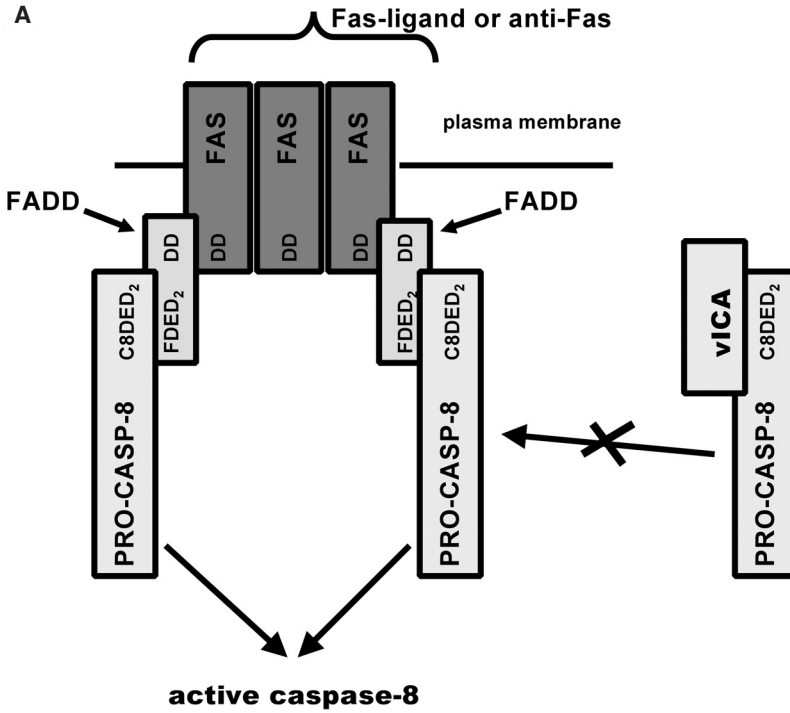
two non-identical death-effector domains (C8DED₂; Skaletskaya et al. 2001). Taken together, these findings provide evidence that vICA directly inhibits proteolytic processing of caspase-8 (Fig. 1), the same step of the death-receptor apoptotic pathway that is blocked by cellular and viral FLIPs (Krueger et al. 2001). FLIPs contain a segment consisting of two non-identical death-effector domains (FDED₂) that is highly homologous to that of pro-caspase-8. FLIPs associate with pro-caspase-8 through C8DED₂-FDED₂ interaction (Krueger et al. 2001). vICA does not share any sequence similarity with FLIPs, and, specifically, it does not contain DED sequence motifs. This dissimilarity between vICA and FLIPs suggests (but does not prove) that vICA and FLIPs block caspase-8 activation by distinct mechanisms. Interestingly, vICA does not associate with FADD (Skaletskaya et al. 2001), an adaptor protein that also has a DED₂ segment in its amino acid sequence. Unlike vICA, c-FLIP is capable of forming a complex with either pro-caspase-8 or FADD (Irmeler et al. 1997). While vICA associates with pro-caspase-8 constitutively in the absence of death-receptor ligation, it appears that under physiologically relevant conditions cFLIP forms a complex with FADD and pro-caspase-8 only at the DISC following ligation of a death receptor (Scaffidi et al. 1999a).

2.3

Homologues of vICA in Other β -Herpesviruses

Searches of DNA sequence databases have thus far failed to reveal any cellular or viral homologues of UL36-encoded proteins. The only exceptions are genomes of other β -herpesviruses. These viruses encode highly conserved vICA homologues (McCormick et al. 2003). Two of these vICA-like proteins, the M36 protein product (M-vICA) of mouse CMV, and the Rh36 protein product (Rh-vICA) of rhesus macaque CMV, were examined and found to be functionally active as cell-death suppressors (McCormick et al. 2003). These data indicate that the ability of vICA to suppress apoptosis is a general function of vICA homologues encoded by β -herpesviruses.

A segment of the guinea pig CMV genome that is, in general, co-linear with the human CMV UL32-UL37 region does not contain a vICA homologue ORF (Liu and Biegelke 2001), an unexpected result, since all other sequenced genomes of β -herpesviruses were found to encode a vICA homologue in this region along with other genes found in human CMV (McCormick et al. 2003). It will be of interest to determine if guinea pig CMV encodes a vICA homologue in another part of its genome, or misses it altogether.



3

vMIA, a Cell-Death Suppressor Targeting Mitochondria

3.1

Cell-Death Suppressing Activity of vMIA

The *UL37* gene of human CMV encodes vMIA, also known as pUL37 $\times$ 1, and its two splice variants, gpUL37 and gpUL37_M. vMIA protects HeLa cells against apoptosis triggered by a wide variety of apoptotic stimuli: ligands of death receptors, various cytotoxic compounds, and infection with an E1B19K-deficient adenovirus mutant (Goldmacher 2002). The longer splice variants of vMIA, gpUL37 and gpUL37_M are, like vMIA, functional cell-death suppressors. The ability of vMIA to protect cells from apoptosis appears to be limited to type II cells (Skaletskaya et al. 2001): vMIA protects HeLa cells and human fibroblasts (both behave as type II cells) from apoptosis, but is inactive as a cell-death suppressor in the type I human lymphoid cell line BJAB. In this respect, vMIA is similar to Bcl-2 that protects type II cells but not type I cells from apoptosis (Foghsgaard and Jaattela 1997; Scaffidi et al. 1998), consistent with the mode of action of Bcl-2, which targets the mitochondrial apoptotic signaling pathway.

3.2

Intracellular Localization of *UL37* Protein Products

Intracellular localization of the three protein products of *UL37*, vMIA, gpUL37, and gpUL37_M, was examined in infected and in transfected cells by immunofluorescence and by electron microscopy (Goldmacher et al. 1999; Colberg-Poley et al. 2000). vMIA is predominantly localized to mitochondria of CMV-infected cells or stably transfected cells, where it decorates the outer mitochondrial membrane. A fraction of vMIA expressed in transiently transfected human cells was observed in the endoplasmic reticulum and the Golgi apparatus (Colberg-Poley et al. 2000). The significance of this phenomenon is at present unclear.

Fig. 1A,B. Two possible models for the cell-death suppressing activity of vICA in Fas-ligation-mediated apoptosis. Ligation of Fas results in recruitment of the adaptor protein FADD [through death domain (DD) homotypic interactions] and pro-caspase-8 [through death effector domain (DED) homotypic interactions] to the death-inducing signaling complex (DISC). Pro-caspase-8 is then processed into active caspase-8. Caspase-8 proteolytically cleaves its substrates such as BID and (in type I cells) pro-caspase-3. vICA binds to the death effector domains of pro-caspase-8 and thus prevents its activation. The precise mechanism of this prevention has not been elucidated. The formation of vICA-pro-caspase-8 complex can either prevent the recruitment of pro-caspase-8 to the DISC (A) or prevent the proteolytic processing of pro-caspase-8 (B)

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