

Dodging the CTL response: viral evasion of Fas and granzyme induced apoptosis

M. Shannon Keckler

Department of Microbiology and Immunology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229

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1. ABSTRACT

The importance of CTL induced apoptosis as a vital part of the protection of host organisms from pathogenic viruses cannot be overstated. Conversely, the ability of a virus to evade CTL induced apoptosis is equally important to its survival. Important insights in viral pathogenesis and host immunology have been discovered through observations of this constantly evolving interchange. This mini review will build upon previously published comprehensive reviews by reorganizing the anti-apoptotic strategies specific for CTL induced apoptosis and integrating recent discoveries in viral evasion of Fas/FasL and perforin/granzyme mediated apoptosis. This updated look at viral evasion in the context of the CTL response should generate dialogue and provide impetus for research to illuminate interactions between the best defense against viruses and the viral adaptations to evade this defense.

2. INTRODUCTION

Comprehensive reviews found in references (1) (2) and (3) have shown a variety of viral proteins that inhibit apoptosis via a diverse set of proteins. This mini review strives to reorganize and update this data to describe viral defenses against a specific piece of host immunity – the CD3+CD8+ cytotoxic T lymphocyte (CTL) response. The CTL response is generally accepted as one of the most important aspects of the immune system for combating viral infections. Briefly, and in general, viral infection of a host cell leads to production of viral proteins in the cytoplasm. These proteins are ubiquitinated, delivered to the proteasome and processed into 8-10 amino acid peptides. These peptides are transported into the endoplasmic reticulum (ER) via the transporter associated with antigen processing (TAP) and loaded onto major histocompatibility complex (MHC) Class I molecules. These MHC:peptide

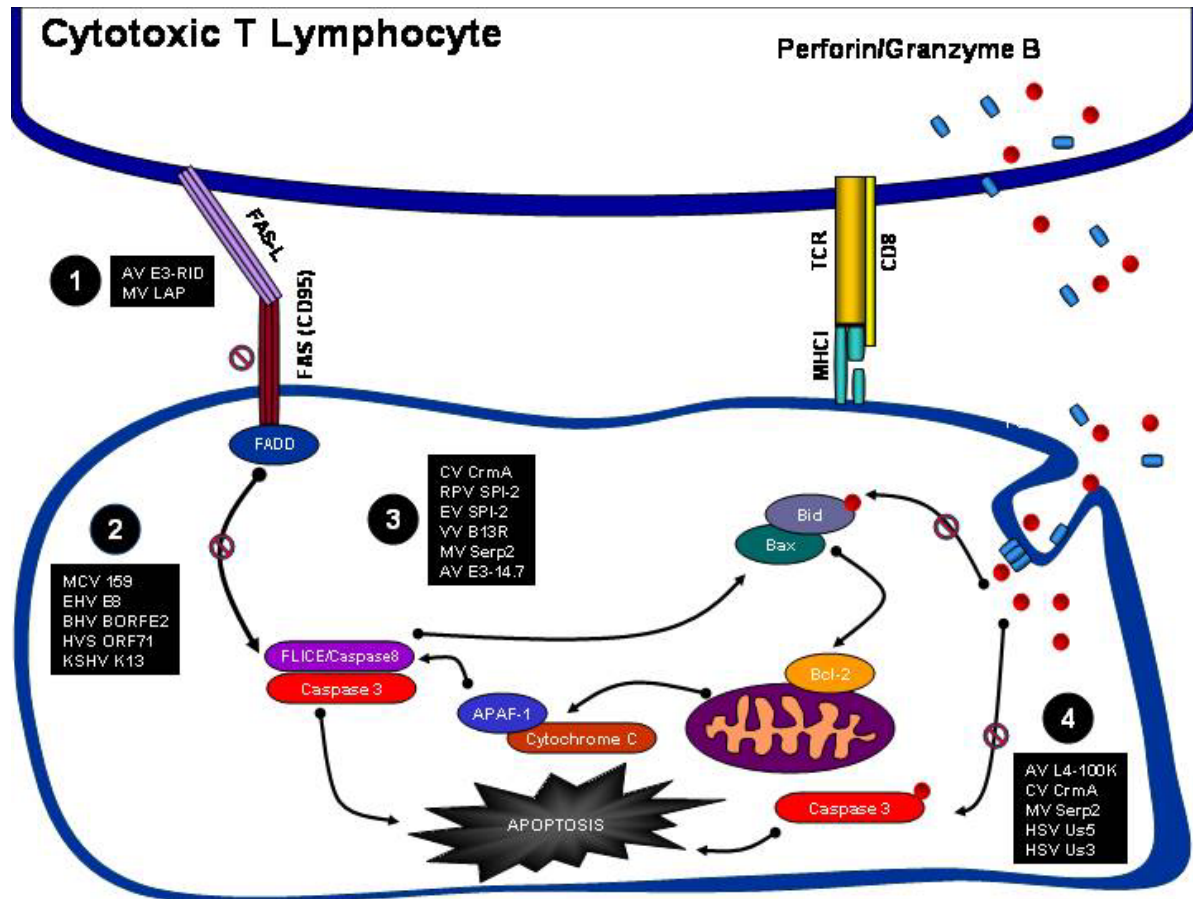


Figure 1. This diagram illustrates the major pathways of CTL induced apoptosis and the viral proteins that are able to block these pathways directly. 1. Viral proteins that are able to downregulate Fas expression or otherwise prevent Fas/FasL binding. 2. vFLIPS and related viral proteins that interfere with FADD/FLICE interaction by interacting with FADD and FLICE. 3. Serpins and related viral proteins that can interfere with FADD/FLICE interaction by interacting with FLICE. 4. Viral proteins that are known to inhibit granzyme B activity either by interrupting granzyme B binding to caspase 3 or interrupting granzyme B binding to Bid.

complexes are processed via the golgi apparatus and exported for display on the cell surface. Circulating T lymphocytes with a T cell receptor (TCR) with complement determining regions (CDR1 and CDR2) that recognize the MHC molecule, and a CDR3 that recognizes the peptide, bind to the infected cell (4). This binding event, coupled with the binding of the CD8 molecule to the MHC molecule, activates the T cell, which causes release of perforin/granzyme and upregulation of Fas/FasL. These two pathways, both of which are described below and shown graphically in "Figure 1", eventually lead to apoptosis of the target cell thus rendering the virus nonreplicative. CTLs are not the only cells that utilize these pathways to induce cell death but they are unique in appearing to have no preference as to which path is utilized. In contrast, NK cells and CD4 Th2 cells prefer the perforin/granzyme pathway and CD4 Th1 cells prefer the Fas/FasL pathway (5).

Understanding the exquisite balance between host cell survival and virus survival requires an understanding of the needs of the virus and the

complexities of the apoptotic pathways. Viruses require the cellular machinery to replicate and spread. If the host cell dies, the virus dies. Because CTL induced apoptosis is an effective way to kill infected cells, it is obvious why many viruses have evolved mechanisms to inhibit CTL induced apoptosis. While there are a multitude of ways that a virus can inhibit apoptosis (1) (2) and (3), this review will be focused on only those virus families that have evolved mechanisms to inhibit CTL induced apoptosis directly via Fas/FasL or perforin/granzyme pathway inhibition. Likewise, there are other ways viruses evade the CTL response that are not included here. For example, those viruses that are able to downregulate MHC Class I molecules (6) (7) or interfere with transport of peptides into the ER (8). Finally, as with any cell signaling phenomena, it is impossible to separate all apoptotic pathways since most of them share components, such as the Caspases and Bcl-2 (5). Therefore, many viral proteins, such as Bcl-2 homologs (9) and effector caspase inhibitors that are able to block CTL induced apoptosis downstream of Fas/FasL signaling and perforin/granzyme activity are not included in this review.

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Although the *inhibition* of CTL induced apoptosis is the focus of this review, it should be noted that many viruses appear to have evolved ways to *induce* apoptosis. While apoptosis induction has not been definitively proven *in vivo* for all viral proteins that show pro-apoptotic activity *in vitro*, and many of the mechanisms of apoptosis induction elude discovery, it seems intuitive that viruses evolved pro-apoptotic proteins to facilitate viral spread. Some viruses, including some of the most currently relevant pathogens such as Coronaviruses (SARS), Filoviruses (Ebola), and Orthomyxoviruses (Influenza), induce apoptosis via a multitude of pro-apoptotic strategies (1,10-18). A brief glimpse of these representative references will serve as an introduction to the many varieties of strategies that viruses employ to *induce* apoptosis. This variety is mirrored in the diversity of mechanisms that have evolved to *inhibit* CTL mediated apoptosis.

3. VIRAL EVASION OF CTL INDUCED APOPTOSIS

As mentioned above, there are two major pathways that are induced by CTL recognition of MHC:peptide complexes on the cell surface. These pathways originate with Fas/FasL binding and perforin/granzyme release. The following is a brief overview of the major players in these CTL induced apoptotic pathways.

One of the consequences of TCR binding to its cognizant MHC:peptide complex is upregulation of Fas/FasL. Binding of FasL on the CTL to Fas molecules on the infected host cell induces the Fas monomers to form trimeric complexes. The intracellular death domain (DD) of the Fas molecule recruits and enables formation of the death inducing signaling complex (DISC) which includes recruitment of the Fas-associated DD (FADD) adaptor molecule. FADD contains an N terminal DD that interacts with Fas and a C terminal death effector domain (DED) that allows interaction with downstream molecules such as Fas-like interleukin (IL)-1 β converting enzyme (FLICE, caspase 8). Interaction with FLICE allows activation of caspase 8 (via cleavage at aspartic acid residues of the regulatory prodomain (5)). Caspase 8, in turn, activates downstream molecules including caspase 3. Activated caspase 3 is able to cleave a variety of substrates including DNA repair enzymes, structural proteins, and caspase-activated deoxyribonuclease (CAD) inhibitor (ICAD) as well as other caspases (19). Activated CAD translocates to the nucleus and causes DNA fragmentation. Although the FADD-FLICE pathway appears to be the major pathway induced by Fas/FasL binding, and is the only one covered by this review, there are alternative CTL mediated pathways, such as the receptor interacting protein (RIP) associated interleukin-1-beta converting enzyme and C. elegans death receptor-3 homolog homologous protein with DD (RAIDD) pathway, that may act as backup pathways (20).

The second major pathway induced by TCR binding to MHC:peptide complexes involves the release of perforin/granzyme. Binding of TCR and MHC:peptide

complex induces polarization of the T cell granules and degranulation of the T Cell. Although the degranulation and host cell uptake mechanisms are not clearly understood (21), it is known that cell lysis can occur via perforin/granzyme pathway when Fas binding is blocked (22). It is also known that perforin and granzyme (primarily granzyme B) are both necessary for CTL induced apoptosis (23). The prevailing model of cell uptake, in which perforin polymerizes at the target cell surface to form a pore through which the granzyme B enters the cell, has been called into question and the newest data seems to indicate that the perforin and granzyme B are endocytosed by the target cell and then perforin acts within the endocytic vesicle to release granzyme B into the cytoplasm (23). Upon entering the cytoplasm granzyme B is able to induce cell death in both a caspase dependent (apoptotic) and a caspase independent (necrotic) manner. granzyme B can activate caspase 3 directly which leads to the activation of CAD and apoptosis. Alternatively, granzyme B can also activate Bid which can lead to activation of BAX which dimerizes with B cell follicular lymphoma 2 (Bcl-2) and removes it from the surface of the mitochondria. This results in a change in the membrane potential and causes destabilization of the mitochondria. Upon destabilization the mitochondria swells and releases cytochrome C, which can cause necrosis of the cell (caspase independent) or can initiate apoptosis (caspase dependent) by binding the apoptotic protease activating factor (APAF-1). This complex then activates caspase 9 which is able to activate caspase 3 (23). Activated caspase 3 is able to initiate apoptosis through CAD.

This summarizes the two major pathways associated with CTL induced apoptosis. The rest of this review will attempt to summarize the viral proteins that are known to inhibit CTL induced apoptosis by blocking the Fas/FasL pathway or the perforin/granzyme B pathway. This review will cover those viral proteins that are capable of interrupting 1) Fas/FasL binding, 2) FADD activation of caspase 8/FLICE, and 3) granzyme B binding of caspase 3 and Bid. "Figure 1" summarizes all of the viral proteins that can directly block CTL induced apoptosis.

3.1. Inhibitors of FAS/FASL binding

As described above the binding of Fas and FasL initiates an apoptotic cascade. Viral proteins have been found that can inhibit the Fas/FasL binding event, providing protection for the virus from apoptosis initiated via the Fas/FasL pathway in CTLs. There are two major ways to inhibit any ligand binding event. The first is to actually block either the receptor (Fas) or the ligand (FasL). The second is to downregulate expression of the receptor or ligand, or render either the receptor or ligand inaccessible. Although tumor necrosis factor receptor (TNFR) homologs have been found in poxviruses (24), there are no known viral Fas homologs that perform a similar receptor blocking or ligand sequestering function. Instead, viruses have evolved mechanisms to block Fas/FasL binding via downregulation of Fas expression. Adenovirus (AV) subgroup C viruses code for an early 3 (E3) region which is known to have multiple anti-immune products (25). The E3-10.4K/14.5K complex specifically induces loss of Fas

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surface expression with no loss of Fas protein production or intracellular stores of Fas (26). Later it was shown that this complex, now called receptor internalization and degradation (RID) complex, mediates this loss of cell surface expression via internalization and subsequent degradation of Fas in lysosomes (27). Although the mechanisms of this are not fully understood, it has been reported that RID β (formerly E3-14.5K) contains a tyrosine residue that is an absolute requirement for Fas downregulation (28) and abrogation of the cell surface localization function of RID α (formerly E3-10.4K) led to deficient Fas downregulation (29). Another AV protein, E3-6.7K, has been shown to be necessary for removal of other receptors from the cell surface by RID but it has not been proven for Fas downregulation (30). A second virus that is known to be capable of Fas receptor downregulation is the Myxoma Virus (MV), which is a poxvirus that infects rabbits. MV leukemia-associated protein (LAP) resides in the ER and has been shown to down-regulate Fas and MHC upon transfection (31). Although this and subsequent studies have illustrated that MV LAP is a ubiquitin ligase that functions to target MHC molecules (32), there are no reports that MV LAP downregulates Fas in the same manner. In addition, there has been no report that the MV LAP homologs found in poxviruses and herpesviruses downregulate Fas receptors. One report indicated that cells infected with Herpes Simplex Virus 2 (HSV-2), but not HSV-1, sequestered Fas within the cell (33), although the viral product responsible for this was never found. There are also many viral proteins that have been shown to downregulate cell surface receptors such as MHC Class I molecules and CD4 molecules and many of these have never been tested for the ability to downregulate Fas.

3.2. Inhibitors of FADD/FLICE

A second way to directly block Fas mediated CTL induced apoptosis is by interfering with the signaling molecules that are recruited by the trimerization of bound Fas/FasL. Multiple viruses have been shown to code for proteins that inhibit the interaction between FADD and caspase 8. One group of these proteins is called viral Fas-associated death-domain-like interleukin-1 β converting enzyme (FLICE/caspase 8) inhibitory proteins (vFLIPs). The best characterized vFLIP is MC159 from Molluscum Contagiosum (MC) virus, another poxvirus. It was originally reported that MC159, MC160 (another MC virus protein) and Equine Herpesvirus 2 (EHV-2) E8 contained 2 DED-like regions. In this same study it was demonstrated that EHV2 E8 interacted with procaspase 8, MC159 interacted with FADD and Fas induced apoptosis was inhibited in the presence of either of these proteins (34). Although MC160 is similar to MC159 in that it shares DED-like regions and the ability to bind FADD, it acts in a cooperative manner with MC159 and is unable to block apoptosis independently (35). Recent research into the mechanism of FADD blocking by MC159 indicates that binding FADD and caspase 8 is not sufficient for apoptosis inhibition but that hydrophobic amino acids in three areas of the MC159 DED-like regions are critical to apoptosis inhibition (36). vFLIPs have also been found in Bovine Herpesvirus 4 (BHV4), Kaposi's Sarcoma-Associated Herpesvirus (KSHV) and Herpesvirus Saimiri (HVS). The

BHV4 BORFE2 shows homology to other vFLIPs and has been shown in a yeast two-hybrid system to interact with procaspase 8 and inhibit apoptosis, (37) indicating that it is most likely similar in mechanism to other vFLIPs. Until recently, vFLIPs were thought to be viral proteins with two DED-like regions that could interact with FADD and FLICE (and other similar proteins in other signaling pathways) in order to block Fas mediated apoptosis. Subsequent research in KSHV and HVS has provided a deeper insight into this group of viral proteins. The HVS vFLIP ORF 71 has been examined *in vivo* and was found to be dispensable for HVS pathogenicity and T cell transformation despite its anti-apoptotic effects (38). This serves as a reminder of the importance of biological context in experimentation and indicates a need for more *in vivo* analysis of the importance of vFLIPs. In addition, KSHV K13 has been shown to prevent caspase activation and was also shown *in vivo* to promote tumor establishment and growth (39). In addition, the KSHV vFLIP has been shown to stimulate high levels of nuclear factor $\kappa\beta$ (NF- $\kappa\beta$) expression via both canonical and non-canonical pathways in primary effusion lymphomas (40) via the physical interaction with and constitutive stimulation of the I-kappa-B kinase (IKK) complex (41) and induction of interleukin-6 (IL-6) (42). The most recent studies indicate that the KSHV vFLIP has oncogenic capabilities in transgenic mice independent of its ability to inhibit apoptosis (43). Based on older work and this collection of recent work on vFLIPs in herpesviruses, it can be concluded that the vFLIP family of proteins are more diverse and multi-functional than was originally thought. Therefore, it may be useful to revisit poxvirus vFLIPS to search for additional functions and to attempt to identify and characterize vFLIPs in the other viral families.

A second group of proteins that interfere with FADD/FLICE are serine proteinase inhibitors (SPI or serpins) that inhibit this interaction by binding to and preventing activation of caspase 8. The first viral protein known to directly block CTL mediated apoptosis by interfering with the Fas/FasL pathway was the Cowpox Virus (CV) cytokine response modifier A (CrmA) protein (44), which had been previously identified as an inhibitor of apoptosis in neuronal cells (45). Subsequently, a homologous serpin in Vaccinia Virus called SPI-2 (B13R) that could also inhibit Fas induced apoptosis by interfering with caspase activation was identified (46) (47). The SPI-2 molecule was also found to inhibit Fas mediated apoptosis in Rabbitpox (RPV) (48) and Ectromelia Virus (EV) (49). The Myxoma Virus (MV) Serp2 was also found to inhibit apoptosis but when tested *in vivo* was not interchangeable with CrmA thus demonstrating the specificity of serpins for particular caspases (50). Using the ability of serpins to specifically inhibit apoptosis, especially CrmA, researchers were able to pinpoint viral protein functional specificity to the FLICE molecule (caspase 8) thus establishing FLICE as a part of the apoptotic pathway (51). Later, caspase 8 assays were useful in finding other viral inhibitors of FLICE. The CrmA story is a prime example of utilizing the interactions between virus and host cell components to help illuminate aspects of each. AV also has a 14.7K protein that is not homologous to any of the serpins but that interacts

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with FLICE and blocked apoptosis in co infection studies (52). More research in this area is needed to clarify the interactions between FLICE and serpins (and other FLICE interacting proteins). It is also possible that there are additional serpins that interact with other caspases that have not been identified.

3.3. Inhibitors of granzyme B

It was first demonstrated in 1995 that granzyme B could bind and activate CCP32 (now called caspase 3) (53). In that same year, CV CrmA was shown to inhibit granzyme B independently of Fas mediated apoptosis (54) as could MV Serp2, relatively weakly when compared to CrmA (55) although the mechanism has not been fully determined. AV assembly protein L4-100K (100K) also inhibits apoptosis by interacting with granzyme B. This interaction is dependent upon an aspartic acid residue found in 100K and is the first known report of a viral protein that is susceptible to cleavage by granzyme B that can inhibit granzyme B (56). The HSV-1 gene Us3 has been shown to interrupt granzyme B pathway by phosphorylation of Bid which prevents granzyme B processing of Bid (57). The HSV-1 Us5 gene has also shown to be important for the inhibition of granzyme B but the mechanism is unclear (58). It is clear that these are only a few of the viruses that would be expected to code for proteins that can disrupt granzyme B mediated apoptosis. More research in this area is needed to increase our understanding of the mechanisms involved in both viral evasion of granzyme B and in the mechanism of granzyme B induced apoptosis.

4. CONCLUSION

There are many factors that should be considered when examining the data associated with viral proteins that inhibit CTL induced apoptosis. First, one should recognize that *in vitro* apoptosis can be induced by many factors inherent in cell cultures so relevant controls and adequate detection assays are vital. In addition, studying Fas/FasL or perforin/granzyme induction of apoptosis without actual CTL stimulation/degranulation removes many confounding factors, such as helper cells and cytokines. This simplifies interpretation of the data but leaves a void between results and biological significance. Also, *in vivo* data cannot always be obtained from an animal model that correlates well with the human disease, which is extremely important when trying to illuminate the workings of complex signaling pathway that exists *in vivo* in a constant state of flux. With those ideas in mind it is easy to see that a multitude of work remains in this area in order to truly understand the mechanisms of apoptosis inhibition. In addition, this review has organized the known and generally accepted data into specific areas where the basic mechanism is proven. This approach excludes a literal gold mine of data that has been published in which Fas mediated apoptosis inhibition has been observed, but the mechanism is simply unknown. For example, it is known that one of the eight Epstein-Barr Virus (EBV) latent genes inhibits apoptosis but which gene is unknown (although EBNA 1 has been ruled out) (59). Pseudorabies virus (PRV) virus blocks apoptosis in nerve cells through another unknown mechanism (60). In African Swine Fever Virus (ASFV) the

gene product EP153R, which codes for a nonessential protein, was shown to be anti-apoptotic *in vitro* using a knockout virus. This is the first description of a viral C-type lectin with anti-apoptotic properties.(61) A better understanding of the anti-apoptotic mechanism and studies of other C-type lectins would be critical to understanding this novel group of anti-apoptotic proteins. A novel anti-apoptotic function for Simian Virus 40 (SV40) T antigen function was discovered when livers of transgenic mice were injected with anti-Fas mAbs. This caused large regions of carcinoma nodules to form that expressed high levels of T antigen (62), indicating a new role for T antigen in SV40 pathogenicity. In addition to these representative examples of experiments that form a basis for additional studies there are also examples of conflicting data in this area that need to be resolved. One such example is Fas-mediated apoptosis in Human Immunodeficiency Virus (HIV) infection. One study has reported that Nef expression delays Fas mediated apoptosis and reduces the number of cells that undergo apoptosis. The cells showed no change in surface expression of Fas but activated caspase 3 and caspase 8 were downregulated in Nef+ cells indicating that Nef plays a role in inhibition of Fas mediated apoptosis. In contrast, when cells expressing HIV proteins were exposed to antigen-specific CTLs there was no inhibition of apoptosis seen, although Nef showed partial protection from CTL recognition, due to its ability to downregulate MHC Class I molecules (63). Further experiments to determine if the results of Nef inhibition of apoptosis are due solely to the downregulation of MHC Class I would be useful. Finally, apoptosis inhibition and/or induction has been observed for almost all vertebrate infecting viral families, but very little understanding of the specific mechanisms has been demonstrated. In addition, studies that concentrate on determining the biological roles of viral inhibition of apoptosis, i.e. as a host range determinant or in pathogenicity studies, are scarce. This review and reorganization of the known data in the specific context of viral evasion of CTL induced apoptosis may lead to further research to increase our understanding of how viruses have evolved to escape this prominent weapon of the host immune system. This difficult and sometimes confusing work is vital to being able to utilize apoptosis inhibition/induction as therapy against viral infections.

5. REFERENCES

1. Roulston, A., R. C. Marcellus & P. E. Branton: Viruses and apoptosis. *Annu Rev Microbiol*, 53, 577-628 (1999)
2. Thomson, B. J.: Viruses and apoptosis. *Int J Exp Pathol*, 82, 65-76 (2001)
3. Benedict, C. A., P. S. Norris & C. F. Ware: To kill or be killed: viral evasion of apoptosis. *Nat Immunol*, 3, 1013-8 (2002)
4. Garcia, K. C. & E. J. Adams: How the T cell receptor sees antigen-a structural view. *Cell*, 122, 333-6 (2005)
5. Waterhouse, N. J. & J. A. Trapani: CTL: Caspases Terminate Life, but that's not the whole story. *Tissue Antigens*, 59, 175-83 (2002)

Dodging the CTL response

6. Servet-Delprat, C., P. O. Vidalain, O. Azocar, F. Le Deist, A. Fischer & C. Rabourdin-Combe: Consequences of Fas-mediated human dendritic cell apoptosis induced by measles virus. *J Virol*, 74, 4387-93 (2000)
7. Poole, B. D., Y. V. Karetyni & S. J. Naides: Parvovirus B19-induced apoptosis of hepatocytes. *J Virol*, 78, 7775-83 (2004)
8. Clarke, P., S. M. Richardson-Burns, R. L. DeBiasi & K. L. Tyler: Mechanisms of apoptosis during reovirus infection. *Curr Top Microbiol Immunol*, 289, 1-24 (2005)
9. Geisbert, T. W., L. E. Hensley, T. R. Gibb, K. E. Steele, N. K. Jaax & P. B. Jahrling: Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses. *Lab Invest*, 80, 171-86 (2000)
10. Li, X. D., S. Kukkonen, O. Vapalahti, A. Plyusnin, H. Lankinen & A. Vaheri: Tula hantavirus infection of Vero E6 cells induces apoptosis involving caspase 8 activation. *J Gen Virol*, 85, 3261-8 (2004)
11. Mendez, E., E. Salas-Ocampo & C. F. Arias: Caspases mediate processing of the capsid precursor and cell release of human astroviruses. *J Virol*, 78, 8601-8 (2004)
12. Sosnovtsev, S. V., E. A. Prikhod'ko, G. Belliot, J. I. Cohen & K. Y. Green: Feline calicivirus replication induces apoptosis in cultured cells. *Virus Res*, 94, 1-10 (2003)
13. Tan, Y. J., B. C. Fielding, P. Y. Goh, S. Shen, T. H. Tan, S. G. Lim & W. Hong: Overexpression of 7a, a protein specifically encoded by the severe acute respiratory syndrome coronavirus, induces apoptosis via a caspase-dependent pathway. *J Virol*, 78, 14043-7 (2004)
14. Gaddy, D. F. & D. S. Lyles: Vesicular stomatitis viruses expressing wild-type or mutant M proteins activate apoptosis through distinct pathways. *J Virol*, 79, 4170-9 (2005)
15. Sharma, K., R. X. Wang, L. Y. Zhang, D. L. Yin, X. Y. Luo, J. C. Solomon, R. F. Jiang, K. Markos, W. Davidson, D. W. Scott & Y. F. Shi: Death the Fas way: regulation and pathophysiology of CD95 and its ligand. *Pharmacol Ther*, 88, 333-47 (2000)
16. Duan, H. & V. M. Dixit: RAIDD is a new 'death' adaptor molecule. *Nature*, 385, 86-9 (1997)
17. Catalfamo, M. & P. A. Henkart: Perforin and the granule exocytosis cytotoxicity pathway. *Curr Opin Immunol*, 15, 522-7 (2003)
18. Henkart, P. A., M. S. Williams, C. M. Zacharchuk & A. Sarin: Do CTL kill target cells by inducing apoptosis? *Semin Immunol*, 9, 135-44 (1997)
19. Lord, S. J., R. V. Rajotte, G. S. Korbutt & R. C. Bleackley: Granzyme B: a natural born killer. *Immunol Rev*, 193, 31-8 (2003)
20. Smith, V. P. & A. Alcami: Expression of secreted cytokine and chemokine inhibitors by ectromelia virus. *J Virol*, 74, 8460-71 (2000)
21. Toth, K., K. Doronin, M. Kuppuswamy, P. Ward, A. E. Tollefson & W. S. Wold: Adenovirus immunoregulatory E3 proteins prolong transplants of human cells in immunocompetent mice. *Virus Res*, 108, 149-59 (2005)
22. Shisler, J., C. Yang, B. Walter, C. F. Ware & L. R. Gooding: The adenovirus E3-10.4K/14.5K complex mediates loss of cell surface Fas (CD95) and resistance to Fas-induced apoptosis. *J Virol*, 71, 8299-306 (1997)
23. Tollefson, A. E., T. W. Hermiston, D. L. Lichtenstein, C. F. Colle, R. A. Tripp, T. Dimitrov, K. Toth, C. E. Wells, P. C. Doherty & W. S. Wold: Forced degradation of Fas inhibits apoptosis in adenovirus-infected cells. *Nature*, 392, 726-30 (1998)
24. Lichtenstein, D. L., P. Krajcsi, D. J. Esteban, A. E. Tollefson & W. S. Wold: Adenovirus RIDbeta subunit contains a tyrosine residue that is critical for RID-mediated receptor internalization and inhibition of Fas- and TRAIL-induced apoptosis. *J Virol*, 76, 11329-42 (2002)
25. Zanardi, T. A., S. Yei, D. L. Lichtenstein, A. E. Tollefson & W. S. Wold: Distinct domains in the adenovirus E3 RIDalpha protein are required for degradation of Fas and the epidermal growth factor receptor. *J Virol*, 77, 11685-96 (2003)
26. Lichtenstein, D. L., K. Doronin, K. Toth, M. Kuppuswamy, W. S. Wold & A. E. Tollefson: Adenovirus E3-6.7K protein is required in conjunction with the E3-RID protein complex for the internalization and degradation of TRAIL receptor 2. *J Virol*, 78, 12297-307 (2004)
27. Guerin, J. L., J. Gelfi, S. Boullier, M. Delverdier, F. A. Bellanger, S. Bertagnoli, I. Drexler, G. Sutter & F. Messud-Petit: Myxoma virus leukemia-associated protein is responsible for major histocompatibility complex class I and Fas-CD95 down-regulation and defines scrapins, a new group of surface cellular receptor abductor proteins. *J Virol*, 76, 2912-23 (2002)
28. Fruh, K., E. Bartee, K. Gouveia & M. Mansouri: Immune evasion by a novel family of viral PHD/LAP-finger proteins of gamma-2 herpesviruses and poxviruses. *Virus Res*, 88, 55-69 (2002)
29. Sieg, S., Z. Yildirim, D. Smith, N. Kayagaki, H. Yagita, Y. Huang & D. Kaplan: Herpes simplex virus type 2 inhibition of Fas ligand expression. *J Virol*, 70, 8747-51 (1996)
30. Bertin, J., R. C. Armstrong, S. Otilie, D. A. Martin, Y. Wang, S. Banks, G. H. Wang, T. G. Senkevich, E. S. Alnemri, B. Moss, M. J. Lenardo, K. J. Tomaselli & J. I. Cohen: Death effector domain-containing herpesvirus and poxvirus proteins inhibit both Fas- and TNFR1-induced apoptosis. *Proc Natl Acad Sci U S A*, 94, 1172-6 (1997)

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31. Shisler, J. L. & B. Moss: Molluscum contagiosum virus inhibitors of apoptosis: The MC159 v-FLIP protein blocks Fas-induced activation of procaspases and degradation of the related MC160 protein. *Virology*, 282, 14-25 (2001)
32. Garvey, T. L., J. Bertin, R. M. Siegel, G. H. Wang, M. J. Lenardo & J. I. Cohen: Binding of FADD and caspase-8 to molluscum contagiosum virus MC159 v-FLIP is not sufficient for its antiapoptotic function. *J Virol*, 76, 697-706 (2002)
33. Wang, G. H., J. Bertin, Y. Wang, D. A. Martin, J. Wang, K. J. Tomaselli, R. C. Armstrong & J. I. Cohen: Bovine herpesvirus 4 BORFE2 protein inhibits Fas- and tumor necrosis factor receptor 1-induced apoptosis and contains death effector domains shared with other gamma-2 herpesviruses. *J Virol*, 71, 8928-32 (1997)
34. Glykofrydes, D., H. Niphuis, E. M. Kuhn, B. Rosenwirth, J. L. Heeney, J. Bruder, G. Niedobitek, I. Muller-Fleckenstein, B.
35. Fleckenstein & A. Ensser: Herpesvirus saimiri vFLIP provides an antiapoptotic function but is not essential for viral replication, transformation, or pathogenicity. *J Virol*, 74, 11919-27 (2000)
36. Sun, Q., S. Zachariah & P. M. Chaudhary: The human herpes virus 8-encoded viral FLICE-inhibitory protein induces cellular transformation via NF-kappaB activation. *J Biol Chem*, 278, 52437-45 (2003)
37. Guasparri, I., S. A. Keller & E. Cesarman: KSHV vFLIP is essential for the survival of infected lymphoma cells. *J Exp Med*, 199, 993-1003 (2004)
38. Liu, L., M. T. Eby, N. Rathore, S. K. Sinha, A. Kumar & P. M. Chaudhary: The human herpes virus 8-encoded viral FLICE inhibitory protein physically associates with and persistently activates the Ikappa B kinase complex. *J Biol Chem*, 277, 13745-51 (2002)
39. An, J., Y. Sun, R. Sun & M. B. Rettig: Kaposi's sarcoma-associated herpesvirus encoded vFLIP induces cellular IL-6 expression: the role of the NF-kappaB and JNK/AP1 pathways. *Oncogene*, 22, 3371-85 (2003)
40. Chugh, P., H. Matta, S. Schamus, S. Zachariah, A. Kumar, J. A. Richardson, A. L. Smith & P. M. Chaudhary: Constitutive NF- κ B activation, normal Fas-induced apoptosis, and increased incidence of lymphoma in human herpes virus 8 K13 transgenic mice. *Proc Natl Acad Sci U S A* (2005)
41. Tewari, M., W. G. Telford, R. A. Miller & V. M. Dixit: CrmA, a poxvirus-encoded serpin, inhibits cytotoxic T-lymphocyte-mediated apoptosis. *J Biol Chem*, 270, 22705-8 (1995)
42. Gagliardini, V., P. A. Fernandez, R. K. Lee, H. C. Drexler, R. J. Rotello, M. C. Fishman & J. Yuan: Prevention of vertebrate neuronal death by the crmA gene. *Science*, 263, 826-8 (1994)
43. Takahashi, A., P. Y. Musy, L. M. Martins, G. G. Poirier, R. W. Moyer & W. C. Earnshaw: CrmA/SPI-2 inhibition of an endogenous ICE-related protease responsible for lamin A cleavage and apoptotic nuclear fragmentation. *J Biol Chem*, 271, 32487-90 (1996)
44. Kettle, S., A. Alcamí, A. Khanna, R. Ehret, C. Jassoy & G. L. Smith: Vaccinia virus serpin B13R (SPI-2) inhibits interleukin-1 β -converting enzyme and protects virus-infected cells from TNF- and Fas-mediated apoptosis, but does not prevent IL-1 β -induced fever. *J Gen Virol*, 78 (Pt 3), 677-85 (1997)
45. Brooks, M. A., A. N. Ali, P. C. Turner & R. W. Moyer: A rabbitpox virus serpin gene controls host range by inhibiting apoptosis in restrictive cells. *J Virol*, 69, 7688-98 (1995)
- Turner, S. J., J. Silke, B. Kenshole & J. Ruby: Characterization of the ectromelia virus serpin, SPI-2. *J Gen Virol*, 81, 2425-30 (2000)
46. Nathaniel, R., A. L. MacNeill, Y. X. Wang, P. C. Turner & R. W. Moyer: Cowpox virus CrmA, Myxoma virus SERP2 and baculovirus P35 are not functionally interchangeable caspase inhibitors in poxvirus infections. *J Gen Virol*, 85, 1267-78 (2004)
47. Zhou, Q., S. Snipas, K. Orth, M. Muzio, V. M. Dixit & G. S. Salvesen: Target protease specificity of the viral serpin CrmA. Analysis of five caspases. *J Biol Chem*, 272, 7797-800 (1997)
48. Chen, P., J. Tian, I. Kovesdi & J. T. Bruder: Interaction of the adenovirus 14.7-kDa protein with FLICE inhibits Fas ligand-induced apoptosis. *J Biol Chem*, 273, 5815-20 (1998)
49. Darmon, A. J., D. W. Nicholson & R. C. Bleackley: Activation of the apoptotic protease CPP32 by cytotoxic T-cell-derived granzyme B. *Nature*, 377, 446-8 (1995)
50. Quan, L. T., A. Caputo, R. C. Bleackley, D. J. Pickup & G. S. Salvesen: Granzyme B is inhibited by the cowpox virus serpin cytokine response modifier A. *J Biol Chem*, 270, 10377-9 (1995)
51. Turner, P. C., M. C. Sancho, S. R. Thoennes, A. Caputo, R. C. Bleackley & R. W. Moyer: Myxoma virus Serp2 is a weak inhibitor of granzyme B and interleukin-1 β -converting enzyme in vitro and unlike CrmA cannot block apoptosis in cowpox virus-infected cells. *J Virol*, 73, 6394-404 (1999)
52. Andrade, F., H. G. Bull, N. A. Thornberry, G. W. Ketner, L. A. Casciola-Rosen & A. Rosen: Adenovirus L4-100K assembly protein is a granzyme B substrate that potentially inhibits granzyme B-mediated cell death. *Immunity*, 14, 751-61 (2001)

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53. Cartier, A., E. Broberg, T. Komai, M. Henriksson & M. G. Masucci: The herpes simplex virus-1 Us3 protein kinase blocks CD8T cell lysis by preventing the cleavage of Bid by granzyme B. *Cell Death Differ*, 10, 1320-8 (2003)
54. Jerome, K. R., Z. Chen, R. Lang, M. R. Torres, J. Hofmeister, S. Smith, R. Fox, C. J. Froelich & L. Corey: HSV and glycoprotein J inhibit caspase activation and apoptosis induced by granzyme B or Fas. *J Immunol*, 167, 3928-35 (2001)
55. Gregory, C. D., C. Dive, S. Henderson, C. A. Smith, G. T. Williams, J. Gordon & A. B. Rickinson: Activation of Epstein-Barr virus latent genes protects human B cells from death by apoptosis. *Nature*, 349, 612-4 (1991)
56. Aleman, N., M. I. Quiroga, M. Lopez-Pena, S. Vazquez, F. H. Guerrero & J. M. Nieto: Induction and inhibition of apoptosis by pseudorabies virus in the trigeminal ganglion during acute infection of swine. *J Virol*, 75, 469-79 (2001)
57. Hurtado, C., A. G. Granja, M. J. Bustos, M. L. Nogal, G. Gonzalez de Buitrago, V. G. de Yebenes, M. L. Salas, Y. Revilla & A. L. Carrascosa: The C-type lectin homologue gene (EP153R) of African swine fever virus inhibits apoptosis both in virus infection and in heterologous expression. *Virology*, 326, 160-70 (2004)
58. Rouquet, N., I. Allemand, T. Molina, M. Bennoun, P. Briand & V. Joulin: Fas-dependent apoptosis is impaired by SV40 T-antigen in transgenic liver. *Oncogene*, 11, 1061-7 (1995)
59. Lewinsohn, D. A., R. Lines, D. M. Lewinsohn, S. R. Riddell, P. D. Greenberg, M. Emerman & S. R. Bartz: HIV-1 Vpr does not inhibit CTL-mediated apoptosis of HIV-1 infected cells. *Virology*, 294, 13-21 (2002)

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Send correspondence to: M. Shannon Keckler, Department of Microbiology and Immunology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229, Tel: 210-258-9853, Fax: 210-670-3329, E-mail: keckler@uthscsa.edu

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