

To kill or be killed: how viruses interact with the cell death machinery

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A virus (from the Latin *virus* meaning toxin or poison) is a small infectious agent that can only replicate inside the cells of another organism. Viruses are found wherever there is life and have probably existed since living cells first evolved. Viruses do not have their own metabolism and require a host cell to make new products. The range of structural and biochemical (i.e., cytopathic) effects that viruses have on the host cell is extensive. Most viral infections eventually result in the death of the host cell. The causes of death include cell lysis, alterations to the cell's surface membrane and various modes of programmed cell death. Some viruses cause no apparent changes to the infected cell. Cells in which the virus is latent and inactive show few signs of infection and often function normally. This causes persistent infection and the virus is often dormant for many months or years. Some viruses can cause cells to proliferate without

causing malignancy, whereas others are established causes of cancer. Human organisms use a genetically controlled cell death programme that prevents the spreading of viral infection and kills the virus. Between 19 and 21 November 2009, with sponsorship from the Journal of Internal Medicine, the Swedish Research Foundation and the Swedish Cancer Society hosted a conference in Stockholm entitled: 'To kill or to be killed. Viral evasion strategies and interference with cell death machinery'. Four comprehensive reviews from this conference are presented in this issue of the Journal of Internal Medicine. These reviews include descriptions of: the modulation of host innate and adaptive immune defenses by cytomegalovirus; the impact of gamma-chain family cytokines on T cell homeostasis in HIV-1 infection and the therapeutic implications; approaches to killing tumours by depriving them of the mechanisms for detoxification; and viral strategies for the evasion of immunogenic cell death.

Keywords: immune response, programmed cell death, virus.

Introduction

Viruses are evolutionarily ancient intracellular parasites that have developed a strategy to use a host cell to replicate and spread. Since the initial discovery of the tobacco mosaic virus by Martinus Beijerinck in 1898, about 5000 of the millions of different types of viruses have been described in detail [1]. Humans use a genetically controlled cell death programme that prevents the spread of the viral infection and kills the virus. Almost 40 years ago, Kerr *et al.* described two modes of cell death, apoptosis and necrosis, and suggested that the former represents an example of gene-regulated processes, whereas the latter is simply a passive event [2]. Since then, the field of cell death has become an increasingly important area of biomedical research. The various modes of cell death

are often defined by morphological criteria without a clear reference to precise biochemical mechanisms. Therefore, in an attempt to clarify the present understanding in cell death research and avoid misinterpretation and/or contradiction, the Nomenclature Committee on Cell Death recently proposed unified criteria for the definition of cell death and its different morphologies [3]. According to this novel classification, twelve cell death modalities (four typical and eight atypical) can be recognized. Typical modes of cell death with relatively well-investigated mechanisms include apoptosis, autophagy, cornification and necrosis. Until recently, the requirement for gene expression was documented only for apoptotic and autophagic cell death. It is interesting that certain genes and their products (e.g., p53, Bcl-2 family proteins, calpains) are important for both these modes of

cell death. Necrosis was long considered to be an example of an accidental and uncontrolled process. Nevertheless, accumulating evidence suggests that necrotic cell death might also be regulated by a specific set of signal transduction pathways and degradative mechanisms. Similar to apoptosis, cell death with a necrotic appearance can contribute to embryonic development and adult tissue homeostasis. Some gene products, such as tumour necrosis factor receptor (TNFR), CD95, TNF-related apoptosis-inducing ligand (TRAIL)-R and receptor-interacting protein (RIP)1, might trigger both apoptosis and necrosis, depending on their interaction with other proteins. Moreover, there is cross-talk between these two cell death modalities. For example, the inactivation of caspases might cause a shift from apoptosis to necrosis, or to a mixture of the two. Recently, the term necroptosis has been introduced to designate a type of programmed necrosis that depends on serine/threonine kinase RIP1 activity [4]. Of interest, it was shown that Bmf, a BH3-only, Bcl-2 family member, is required for death receptor-induced necroptosis. Indeed, it seems that Bcl-2 family proteins are essential for the regulation of the majority of programmed cell death modalities.

The cell death-related terminology proposed by the Nomenclature Committee also includes the following atypical modes of cell death: mitotic catastrophe, which can lead either to apoptosis or necrosis; anoikis; excitotoxicity; Wallerian degeneration; paraptosis; pyroptosis; pyronecrosis; and entosis. Although they are all characterized by distinct morphologies, the molecular mechanisms underlying the majority of these cell death modes have not been thoroughly investigated.

Viruses and cell death programmes

Apoptotic cell death is accompanied by the activation of cysteine proteases, caspases. However, it also might be caspase-independent. Both mechanisms are controlled by anti-apoptotic members of the Bcl-2 family proteins as well as by inhibitor of apoptosis proteins (IAPs). Many viruses encode anti-apoptotic proteins that allow them to complete viral replication before destruction of the cell and spread of the virus. Therefore, apoptotic cell death plays a central role in host defense by blocking viral expansion. However, apoptosis might also facilitate viral egress, and the suppression of apoptosis could inhibit viral pathogenesis. Thus, apoptotic cell death evoked by viruses has a complex role in host defense and might facilitate the clearance of viruses or serve as a mechanism for

virus-induced tissue injury and progression of disease. The diseases evoked by viral infections might be associated with a reduction of apoptosis (e.g., infections induced by adenoviruses, baculoviruses, herpesviruses and poxviruses). In contrast, however, some might be associated with increased apoptosis (e.g., infections induced by Ebola or human immunodeficiency virus (HIV)-1) (Fig. 1). In addition to apoptosis, viral infection might induce the other modes of cell death, or even their combination. For example, sindbis virus infection of motor neurons causes cell loss in the spinal cord through necrosis but also induces the classical apoptosis of cortical neurons in the brain [5], suggesting a cell type-dependent response to the same virus.

Viral infection might also be associated with autophagy, a process essential for degrading the bulk of the cytoplasm, including proteins and organelles, and a mechanism for engulfing entire virus particles. Some viruses [e.g., HSV-1, Kaposi's sarcoma-associated herpesvirus (KSHV), mouse herpesvirus 68 (MHV-68)] encode proteins that block the autophagic pathway through interaction with the autophagy-related protein Beclin-1, thereby protecting viruses from degradation [6]. In some cases, autophagy might precede apoptosis. Thus, HIV-1 envelope glycoprotein (Env)-mediated autophagy in bystander CD4⁺ T lymphocytes leads to apoptosis, and cells with inhibited apoptosis undergo cell death with autophagic features [7]. In contrast, the inhibition of autophagy by the influenza virus via a viral matrix protein 2 facilitates the execution of apoptosis in virus-infected cells [8].

The infection itself is initiated by binding of the virus to the host cell surface receptor(s) and penetration of the plasma membrane. Thus, HIV-1 requires the presence on the surface of T cells of CD4, CC-chemokine receptor 5 and CXCR4-chemokine receptor 4. Some viruses, such as poxviruses, can enter the cell without binding to any obvious receptors at the cell surface; for example, the prototype poxvirus vaccinia virus uses macropinocytosis to enter the host cell. The presence of phosphatidylserine at the surface of the viral membrane was suggested to be important for the induction of blebs and infection of the host cell by vaccinia virus [9]. The first line of defense, which constitutes the innate immune response, is activated soon after the infection of the host [10] and the second line, represented by cells of the adaptive immune system (CD4⁺, CD8⁺ T and B cells), is stimulated by antigen-presenting cells (APCs). The host APC digests viral proteins, providing antigenic peptides and cou-

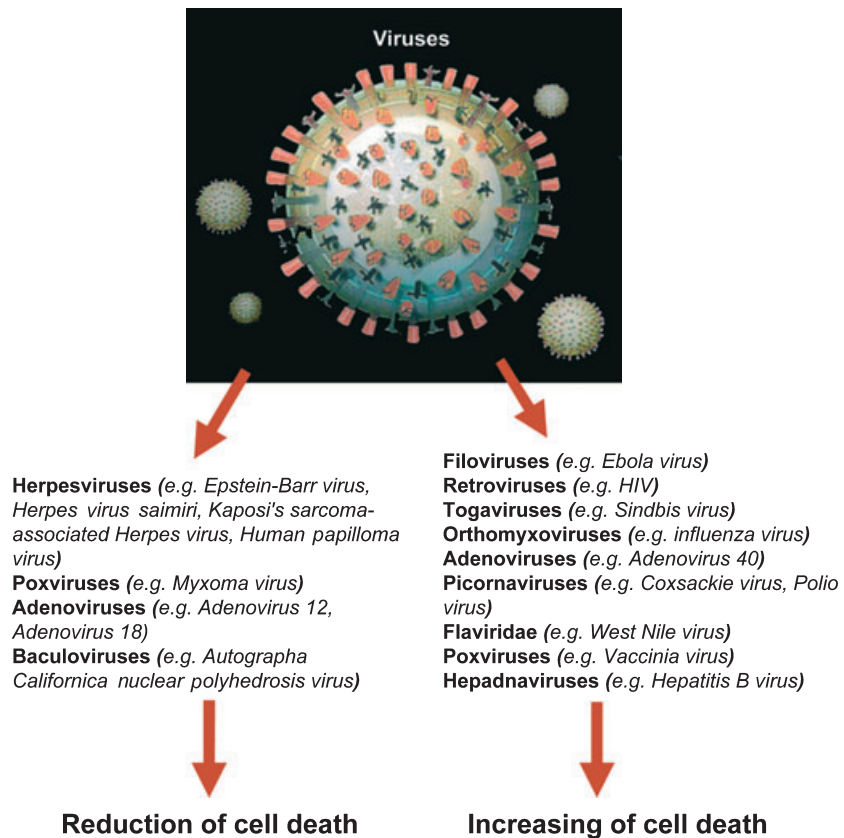


Fig. 1 Cell death-related consequences of viral infection.

pling them to the major histocompatibility complex (MHC), thereby leading to the activation of the adaptive immune response [11].

Antiviral mechanisms are initiated by several pattern recognition receptors (PRRs) expressed in most somatic cells. These PRRs include RNA-dependent protein kinase (PKR) and/or general control non-repressible-2 (GCN2), which lead to the downregulation of mRNA translation [12], 2'-5'-oligoadenylate synthetase (2'-5' OAS), which activates RNase L-inducing RNA degradation, and adenosine deaminase acting on RNA (ADAR-1), which deaminates adenosine on a molecule of double-stranded RNA (dsRNA). The resulting signalling, which is triggered by infection, induces the secretion of several cytokines including interferons (IFNs), tumour necrosis factor (TNF) and immunoregulatory interleukins (ILs) [13].

IFNs and cell death

Interferons are secreted cytokines that exhibit antiviral, antiproliferative, antitumour and immunomodulatory

properties. There are two main pathways (endosomal and cytosolic) within the cells leading to IFN gene expression. In the endosomal pathway, a virus is taken up by the cell enclosed in an endosome and then recognized by several Toll-like receptors (TLRs) and via the adaptor proteins MyD88 or TRIF, or via TRIF and TBK-1, IKK ϵ phosphorylates and activates transcription factors interferon regulatory factor (IRF), thus inducing the expression of IFN- α and IFN- β . In the cytosolic pathway, viral RNA or DNA is recognized by different PRRs and, through the activation of kinases TBK-1, IKK ϵ activates IRF, which activates the expression of IFNs. INF signalling mediated through the c-Jun N-terminal kinases/stress-activated protein kinase (JAK/STAT) pathway leads to the activation of IFN-stimulated genes. To date, it has been found that up to 300 genes are induced following treatment with IFN, and it has been established that several of these genes are involved in cell death. There are several distinct pathways linking IFNs and cell death; however, the basic mechanism of their action is still unclear. For instance, some of these genes, such as PKR or OAS, are activated by viral dsRNA and affect apoptosis [14, 15]. The activation of PKR

leads to the phosphorylation of the α subunit of the translation initiation factor eIF-2 (eIF-2 α), which inhibits protein synthesis and is involved in apoptosis [16]. Although IFN- α/β signalling by itself does not activate pro-apoptotic p53 protein, it has been shown that the p53 response in virus-infected cells could be enhanced by IFN- α/β . It is noteworthy that the absence of p53 makes vesicular stomatitis virus- or encephalomyocarditis virus-infected cells more resistant to IFN-induced apoptosis [17]. It is also known that some procaspase genes are IFN inducible. The antiviral response of natural killer (NK) cells is activated by IFN through the induction of receptor-mediated apoptosis by TRAIL. Infected cells are susceptible to TRAIL-mediated cytotoxicity *in vitro* and, furthermore, TRAIL expressed in NK cells is crucial for limiting viral replication *in vivo* [18]. Recently, it was shown that another IFN-inducible protein, AIM (absent in melanoma 2), senses cytoplasmic DNA and interacts with ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain), leading to the activation of caspase-1 and inducing pyroptotic cell death [19]. Thus, induction of the IFN system establishes a mechanism of host defense by clearing the virus-infected cell and, therefore, inhibiting the spreading of viral infection.

Granzymes kill virus-infected cells

A response to viral infection also involves killing the infected cells with cytotoxic lymphocytes [cytotoxic T lymphocytes (CTLs) and NK cells], and protecting human cells against viral infection in a perforin-dependent manner by delivering serine proteases (granzymes) into the target cell. There are five different granzymes expressed in human cells and the most abundant of these are granzyme A and granzyme B [20]. Several different granzymes have been shown to activate specific cell death pathways and some of them may have evolved to interfere with the replication of certain pathogenic viruses [21]. Thus, granzyme H might cleave and inactivate two adenoviral proteins – DNA-binding protein (DBP) and the L4-100K assembly protein – thereby significantly restricting viral replication and preventing the virus from blocking the potent pro-apoptotic activity of granzyme B [22]. Granzyme B can promote caspase-dependent cell death through the direct cleavage of substrates including caspase-3, -6, -7, -8, -9 and -10 or use caspase-independent apoptosis by triggering mitochondrial permeabilization or by cleavage of DFF45/inhibitor of caspase-activated DNase (ICAD) [23]. It is interesting that resting murine NK cells express large amounts of granzyme A but have low

cytotoxicity. However, the activation of NK cells in response to the cytomegalovirus (CMV) induced translation of granzyme B and perforin mRNAs, thus promoting the potent cytotoxicity of NK cells [24]. Therefore in response to viral infection, in addition to the cytokine defense system, humans use killer cells to trigger death in infected cells by delivering lethal proteases into the target cell.

Viral suppressors of cell death

Viruses evolved a strategy to inhibit apoptosis by encoding a number of suppressors that block intrinsic and extrinsic host-initiated cell death pathways. A number of adenoviruses, herpesviruses, poxviruses and CMVs encode proteins that are homologous to the cellular anti-apoptotic Bcl-2 family of proteins. The adenoviral protein E1B-19K was the first viral Bcl-2 homologue to be discovered. It sustains viral replication by blocking host cell apoptosis via the sequestration of pro-apoptotic Bcl-2 family members Bak, Bax and Bnip3. Adenovirus-infected cells are protected from cell death induced by various stimuli including growth factor deprivation, E1A-triggered activation of p53, Fas, TNF- α and TRAIL death ligands [25].

The human CMV also encodes the viral mitochondria-localized inhibitor of apoptosis (vMIA), which is targeted to mitochondria to suppress cell death by binding to and inhibiting Bax [26]. vMIA also counteracts serine protease HtrA2/Omi (High temperature requirement protein A2/Omi stress-regulated endonuclease)-dependent cell death and allows infected cells to survive and continuously produce a virus for several days [27]. Recently, a small mitochondria-localized protein encoded by the murine CMV open reading frame (ORF) m41.1 or by other CMVs has been described and found to function as a viral inhibitor of Bak oligomerization (vIBO) [28]. To prevent apoptosis induced by various stimuli, CMV employs additional proteins [including pUL38, IE1(491a) and IE2(579aa)] and the viral RNA beta2.7, which bind to the mitochondrial respiratory complex I, maintaining ATP production late in infection and preventing death induced by mitochondrial poison [29].

Gamma herpesviruses, including Epstein-Barr virus (EBV), KSHV (also known as HHV-8), herpesvirus saimiri (HVS) and the murine γ herpesvirus 68 (γ HV68), also encode a homologue of Bcl-2. EBV is a human virus that infects epithelial and lymphoid cells and leads to cell transformation. The viral BHRF1 gene ex-

presses a Bcl-2 homologue protein that resembles Bcl-2 in its subcellular localization and capacity to enhance B cell survival [30]. BHRF1 was shown to inhibit apoptosis induced by growth factor withdrawal, granzyme B, γ -irradiation, chemotherapeutic drugs, deregulated c-Myc and p53 [31–34]. KSHV is associated with the development of human cancers, and Kaposi's sarcoma Bcl-2 shares homology in BH1 and BH2 domains with Bcl-2 [35]. Similar to BHRF-1, Kaposi's sarcoma Bcl-2 is expressed early in the lytic replication cycle and can inhibit apoptosis induced by various stimuli. In addition to the apoptosis-inhibiting functions of vBcl-2, it has been suggested that evasion of autophagy-mediated host innate immunity serves as a key aspect of γ HV68 replication and the pathogenesis of murine γ HV68 [36]. The vaccinia virus protein F1L inhibits Bak or can indirectly inactivate Bax because of its interaction with the Bax activator Bim, thereby inhibiting cytochrome c release induced by diverse stimuli.

The spreading of viral infection is inhibited by CTLs by recognizing and killing infected cells via death receptor-mediated mechanisms. Several herpesviruses, including KSHV and HVS, contain viral FLICE-inhibitory proteins (FLIPs). Death effector domains of viral FLIPs interact with the adaptor protein fas-associated via death domain, and this inhibits the CD95 death receptor-mediated recruitment and activation of caspase-8 and -10 [37].

Inhibitor of apoptosis proteins inhibit cell death in response to a wide variety of stimuli. Viral IAPs (vIAPs) are encoded by baculoviruses and certain poxviruses. All vIAPs contain a carboxyl ring finger and a variable number of highly conserved Cys/His motifs known as baculoviral IAP repeats (BIRs). The BIR domains bind directly to caspases and inhibit their proteolytic activity [38], and molecules that contain an IAP-binding motif (second mitochondrial-derived activator of caspases and Omi) antagonize IAP function via binding to BIR motifs, displacing IAP binding to caspases or by promoting their degradation [39]. Therefore, vIAPs act downstream of mitochondria, inhibiting the activity of procaspase-9 and effector caspase-3 and -7 [40].

Many poxviruses encode homologues of human serpins, which are a family of serine proteinase inhibitors. Poxviruses are the only viruses so far discovered that express functional serpins. CrmA is one of the best known serpins. It inhibits apoptosis triggered by TNF or the ligation of Fas receptors due to inhibition of caspase-8 or caspase-1, which is involved in the

inflammatory response. In addition, crmA has some activity against the serine proteinase granzyme B [41].

Viruses are also able to inhibit IFN-mediated antiviral action. Indeed to survive viral infection, several mechanisms have evolved to inhibit PKR activity (IFN-inducible gene), such as adenovirus VAI RNA, vaccinia virus K3L protein, hepatitis C NS5A protein and influenza virus-induced p58 protein, which interact with PKR to impede its ability to bind to target substrates. The herpes simplex virus γ _{134.5} protein directs the cellular protein phosphatase 1 to dephosphorylate eIF-2 α , whereas poliovirus employs a cellular proteinase to degrade PKR [42]. Some IFN-inducible genes, such as those of the MHC, are controlled by CMV following host infection. Finally, some viruses, such as adenovirus E1 and Sendai virus, target the IFN-inducible JAK/STAT pathway, whereas others, such as vaccinia virus, encode decoy IFN receptors [43].

Viral transcription factors control the expression of a number of genes that counteract the pro-apoptotic functions of p53. For example, EBV infection induces the hyperphosphorylation of pRb, p107 and p130 and leads to E2F family member expression and cyclinE/cdk2 complex activation, driving infected cells into the S phase where p53 is stabilized. The virus does not directly interact with p53, but it nonetheless interferes with cell-cycle checkpoints regulated by p53 in the G1/S and G2/M phases and modulates p53-dependent apoptosis [44, 45]. Some viruses encode proteins that directly interact with p53 or modulate its function. For example, HPV-16 encodes the E6 protein that in combination with the ubiquitin ligase E6AP forms a complex that specifically targets p53 for ubiquitin-mediated degradation [46], and HBx (hepatitis B virus protein) and adenoviral protein E1B-55K bind to p53 and inhibit its transcriptional activity [47]. Simian virus 40 protein (SV40-LT) binds to the DNA-binding domain of p53 and prevents the activation of p53-dependent genes [48]. Thus, by utilizing several mechanisms depending on the types of infected cells, viruses can suppress the cell death process often leading to uncontrolled cell survival.

Viral proteins that induce apoptosis

Apoptosis of virus-infected cells is induced either by stimulating the immune response or by introducing viral suicide genes that kill the host cell. Significant cell death during HIV-1 infection can be attributed to the direct viral killing of infected peripheral blood

mononuclear cells in AIDS patients. HIV-1 kills CD4-expressing primarily infected cells, such as CD4⁺ T helper cells, T lymphocytes, monocytes and dendritic cells as well as bystander cells through cell-exposed proteins (such as Env) or secreted proteins (including Vpr, Tat, Nef, Vpu, Vif and the viral protease) [49]. Different molecular targets are implicated in the cell death induced by HIV-1 proteins. Thus, Env triggers apoptosis through the activation of the mammalian target of rapamycin (mTOR) and p38 MAP kinase, and transactivation of the p53-dependent genes PUMA and Bax [25]. Several mechanisms are thought to be involved in cell death induced by HIV-1 proteases, such as the protease-mediated activation of caspase-8 or proteolytic cleavage of Bcl-2 [50, 51]. Another HIV-1 protein, Vpr, has been shown to directly interact with adenine nucleotide translocase and voltage-dependent anion channel, triggering mitochondrial membrane permeabilization and the activation of the caspase cascade [52]. In contrast, Tat induces the alteration of microtubule dynamics and leads to the activation of a mitochondrial-dependent apoptotic pathway promoted by Bim [53].

An additional disease that is associated with increased cell death evoked by the highly pathogenic Ebola virus can induce haemorrhagic fever syndrome in humans. The disease evoked by the virus is characterized by the release of plasma inflammatory cytokines including IL-1 β , IL-6 and TNF α ; however, the late stage of infection is associated with a poor immune response [54]. Indeed, in infected dendritic cells, the Ebola virus inhibits the secretion of pro-inflammatory cytokines, but does not induce costimulatory molecules, and impairs the adaptive immune response [55]. The virus encodes proteins VP35 and VP24, which block IFN production and inhibit signalling downstream of the IFN- α/β and IFN- γ receptors. The question of how the Ebola virus induces massive apoptosis of lymphocytes remains unresolved because this virus does not target these cells directly; however, the number of lymphocytes is rapidly reduced once the virus is detected in the organism. In fact, experiments in which macaques were infected with Ebola virus showed that lymphocytes remained free of viral infection whereas NK, CD4⁺ and CD8⁺ cells underwent massive apoptosis during the course of the disease [56]. It was suggested that inflammatory mediators or nitric oxide secreted by infected macrophages are capable of inducing bystander cell death or that viral proteins might induce lymphocytic cell death [57]. Thus, some pathogenic viruses have developed a killing strategy by introducing suicide genes into the cells and implicating

different molecular death pathways (i.e., encoding proteins that directly permeabilize the mitochondrial membrane), transactivating pro-apoptotic genes such as PUMA and Bax or inhibiting anti-apoptotic Bcl-2 family members.

Other modes of cell death induced by viral infection

As mentioned above, several types of viral infection have been shown to induce necrosis. In contrast to the finding that HIV-1 induced apoptotic cell death, it was demonstrated that HIV-1-mediated killing of some T cells did not depend on caspases and apoptosis. In this situation, cell death induced by HIV-1 lacks some hallmarks of apoptosis, such as the externalization of annexin V, caspase activity or DNA fragmentation, and appears to be necrotic [58, 59]. Infection with coxsackievirus (an enterovirus belonging to the *Picornaviridae* family) also induced necrotic-like cell death as characterized by cell swelling followed by loss of membrane integrity [60]. In addition, West Nile virus infection was shown to induce necrosis that was accompanied by leakage of a pro-inflammatory mediator cytokine, the high mobility group 1 (HMGB1) protein, into the extracellular space. The viral effect on membrane integrity was suggested to be due to budding of virus particles during maturation [61]. Vaccinia virus-infected cells with inhibited caspase-8 underwent programmed necrosis in response to TNF that was rescued by a deficiency of serine/threonine protein kinase RIP [62]. Recent data have shown that vaccinia virus infection induced programmed necrosis via the activation of RIP3, which regulates necrosis-specific RIP1 phosphorylation, triggering the activation of pro-necrotic kinase activity and the formation of reactive oxygen species (ROS). The activation of necrosis is important for virus-induced inflammation and innate immune control of viral infections [63].

Autophagy in virus-infected cells might be accompanied by other modes of cell death, or it might be involved in the sensitization of infected cells to apoptosis or have an inhibitory effect on apoptotic cell death that has been evoked by viral infection. For example, autophagy mediated by HIV-1 Env is required to trigger T cell apoptosis. Thus, autophagy precedes virus-infected apoptotic cell death. However, Env-mediated cell death with autophagic features occurred in T cells with inhibited apoptosis, indicating that in this case autophagy alone might account for the entire cell killing process [7]. Several types of viral infection, such as poliovirus, coxsackie B virus and hepatitis C virus,

induce the initial stages of autophagy (i.e., the formation of LC3-positive autophagic vesicles) but not the late stages or lysosomal degradation observed in infected cells [6]. It was recently found that HIV-1 protein Nef interacts with the autophagy-related protein Beclin-1, thereby guarding virions from autophagic elimination and extending the life span of macrophages [64]. It has also been shown that apoptosis increases as a result of the inhibition of macroautophagy (autophagic pathway in which the formed autophagic vesicles fuse with lysosomes for the degradation of autophagosomal contents) in cells infected with the influenza A virus [8]. The virus encodes matrix protein 2, which blocks the fusion of autophagosomes with lysosomes and thereby significantly increases apoptosis of influenza-infected cells. Thus, virus-initiated apoptosis or necrosis is subsequently followed by the death of the infected cell. In contrast, autophagy evoked by viral infection might preserve cell survival or promote cell death depending on either type of virus or type of infected cell.

Conclusion

The recent conference in Stockholm, entitled: 'To kill or to be killed. Viral evasion strategies and interference with cell death machinery' integrated for the first time the two fields of viral pathogenesis and cell death (apoptosis and autophagy), to combine the research efforts in these areas and discuss potential therapeutic and vaccine strategies. During the meeting, there was an interesting discussion of the events occurring during viral infection and viral pathogenesis that lead to the activation/inhibition of various molecular pathways of cell death.

The dysregulation of cell death mediated by virus and viral proteins has become an important and interesting target for the development of new therapies aimed at controlling and reversing the pathogenesis of diseases triggered by viral infection. Pathogenesis induced by viral interference with cell death pathways is important for the development of vaccination strategies.

In this issue, four review articles from this conference are included. A. Loewendorf and C.A. Benedict suggest several strategies for the modulation of host innate and adaptive immune defenses by CMV [65]. These strategies are presented in the context of the period of greatest effect on host defence, including timing and tissue-specific issues during the spreading of CMV. As CMV infects more than 50% of the global population, a high priority for the scientific com-

munity is the development of an anti-CMV vaccine for use in preventing congenital infection.

M.-L. Gougeon and F. Chiodi summarize the role of gamma-chain cytokines, especially IL-2 and IL-7, in influencing T cell homeostasis and proliferation and discuss how immunotherapy with these cytokines might be beneficial to reconstitute the T cell compartment in the context of HIV-1 infection [66]. Two large trials evaluating the efficacy of IL-2 in restoring immune function during HIV-1 infection are being performed and are showing intriguing results. The promising results and caveats from the first phase I/II clinical trials with IL-7 in HIV-1-infected patients are summarized in this interesting article, along with the authors' view of the knowledge that is still lacking in the field of T cell reconstitution through gamma-chain cytokines.

S. Fais presents a novel approach to killing tumours by depriving them of the mechanisms for detoxification [67]. Based on early evidence, this approach considers the vATPase to be an important target because of its crucial function in determining the acidification of the tumour microenvironment and consequently the elimination of toxic molecules (such as H⁺ or ROS). By treating tumour tissues with H⁺ pump inhibitors, cancer cells can be specifically killed by a sudden and devastating cell death mechanism selectively induced by intracellular H⁺ accumulation. How successful this approach might be will become apparent in the near future.

Viral strategies for the evasion of immunogenic cell death are discussed by Galuzzi *et al.* [68]. In several recent publications, this group has attempted to reveal the molecular pathway that underlies immunogenic cell death. This type of death can be divided into three major components: 'ER stress', 'apoptotic' and 'CRT/ERp57 co-translocation' modules. Molecular tools that specifically target the machinery for immunogenic cell death have provided viruses with an additional means of evading host immune response, and thereby an evolutionary advantage. In view of this, the CRT/ERp57 exposure, which delivers an 'eat me' signal that facilitates engulfment by dendritic cells, represents a promising target for the development of novel antiviral agents with an aim of enhancing the immunogenicity of cells succumbing to viral infection.

It is interesting that using the potential capacity of some viruses (e.g., alpha viruses) to induce apoptosis, and using these viruses as vectors for gene

therapy in the treatment of certain cancers has recently been suggested [69]. In fact, such an attempt has been made with sindbis virus vectors, showing that this approach is feasible [70]. However, more extensive genetic engineering and preclinical testing is needed to develop this idea and produce an effective anticancer drug.

It is clear that a deeper understanding of the molecular mechanisms of viral infection and the interaction between viruses and different cell death programmes that lead to various diseases will result in the development of novel and improved treatment protocols. Indeed, several agents that are now in clinical trials may become valuable additions to the future therapeutic arsenal, which will probably include a number of cytotoxic drugs that can be used in combination with molecular target-based pro-cell death drugs. Such novel drugs that can be used successfully in disease therapy are eagerly awaited.

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Conflict of interest statement

No conflict of interest was declared.

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