Caspases leave the beaten track: caspase-mediated activation of NF-κB

Mohamed Lamkanfi, Wim Declercq, Tom Vanden Berghe, and Peter Vandenabeele

Unit of Molecular Signaling and Cell Death, Department for Molecular Biomedical Research, Flanders Interuniversity Institute for Biotechnology (VIB), Ghent University, B-9052 Ghent, Belgium

The proteolytic activity of the cysteinyl aspartate–specific proteases, named caspases, mainly connotes their central role in apoptosis and inflammation. In this review we report on recent data on the role of caspases in the activation of nuclear factor κB (NF-κB), a transcription factor that fulfils a central role in innate and adaptive immunity, in cellular stress responses and in the induction of anti-apoptotic factors. Two different mechanisms by which caspases activate the NF-κB pathway are discussed.

With the notable exceptions of the well-documented roles of caspase-1 (Li et al., 1995; Ghayur et al., 1997) and -11 (Wang et al., 1998) in the processing and release of interleukin-1β (IL-1β) and interleukin-18 (IL-18), members of the caspase family have mainly been associated with the initiation and execution of apoptosis (Lamkanfi et al., 2003). However, several recent reports have further substantiated the nonapoptotic roles of these so-called apoptotic caspases.

Caspases are synthesized as zymogens with a prodomain of variable length followed by a large and a small subunit (for review see Lamkanfi et al., 2003). The large prodomains contain a class of related protein recruitment motifs, such as the caspase recruitment domain (CARD, caspases-1, -2, -4, -5, -9, -11, and -12) and the death effector domain (caspase-8 and -10). These prodomains allow recruitment in large protein complexes, eventually leading to caspase auto-activation and initiation of the apoptotic and inflammatory pathways (Fig. 1). That is why large prodomain-containing caspases are often referred to as initiator caspases. These complexes consist of a platform protein that senses a trigger and that can either directly or by means of adaptors recruit specific large prodomain caspases. The death-inducing signaling complex (DISC) containing caspase-8 is formed at the intracellular domain of death receptors, such as Fas, TRAIL receptor, and TNF receptor 1 (Peter and Krammer, 2003; Varfolomeev et al., 2005). Fas-associated death domain-containing protein (FADD) plays a crucial role in the recruitment and activation of caspase-8 and -10 in the DISC. Mitochondrial damage results in release of cytochrome *c*, triggering the assembly of the apoptosome complex that directly recruits caspase-9 (Cain et al., 2002). In vitro, caspase-2 is part of a high molecular weight complex containing the p53-induced death domain protein (PIDD) (Tinel and Tschopp, 2004). Because the p53 tumor suppressor can elicit apoptosis in response to DNA damage, it was suggested that this PIDDosome complex is formed under DNA-damaging conditions and functions as a platform for caspase-2 activation. Last but not least, the inflammatory caspases-1 and -5 have also been shown to be recruited to a number of protein platforms, named inflammasomes (Martinon and Tschopp, 2004). Typically, the platform of these complexes consists of members of the NACHT-LRRs (NLRs) (Martinon and Tschopp, 2005). NLRs are intracellular pathogen-recognition receptors that initiate inflammatory signaling and/or cell death. These intracellular receptors are activated by different pathogen-associated molecular patterns or PAMPS such as bacterial RNA, lipopolysaccharide (LPS), or peptidoglycans.

Generally, recruitment of large prodomain caspases in these complexes induces a conformational change that is sufficient to activate these caspases, leading to autoproteolysis (Boatright and Salvesen, 2003). The short prodomain caspases are activated by proteolytic maturation by initiator caspases or other proteases, and are referred to as executioner caspases (caspases-3, -6, -7, and -14). The final outcome of these proteolytic cascades is the specific cleavage of a wide variety of substrates that are implicated in apoptosis and inflammation. Here, we discuss recent data that implicate caspases in nuclear factor κB (NF-κB) activation pathways. These nonapoptotic caspase functions involve both prodomain-mediated NF-κB activation, depending on recruitment of factors that initiate NF-κB activation, and a mechanism involving limited proteolytic activity of caspases. The latter mechanism relates to complex-mediated caspase activation that considers only a small fraction of the total cellular pool of a particular caspase. Under such conditions the initiator caspases remain associated with the initiating complex.

NF-κB is a dimeric transcription factor involved in immune regulation, apoptosis, cell proliferation, differentiation,

Correspondence to Peter Vandenabeele: peter.vandenabeele@dmbr.ugent.be

Abbreviations used in this paper: CARD, caspase recruitment domain; DISC, death-inducing signaling complex; FADD, Fas-associated death domain-containing protein; IKK, IκB kinase; IL, interleukin; LPS, lipopolysaccharide; NF-κB, nuclear factor κB; NLR, NACHT-LRR; PARP, poly(ADP-ribose)polymerase; PIDD, p53-induced death domain protein, RIP, receptor interacting protein; TcR, T-cell receptor; TLR, toll-like receptor; TRAF, tumor necrosis factor-receptor associated factor.

The Journal of Cell Biology, Vol. 173, No. 2, April 24, 2006 165–171
http://www.jcb.org/cgi/doi/10.1083/jcb.200509092

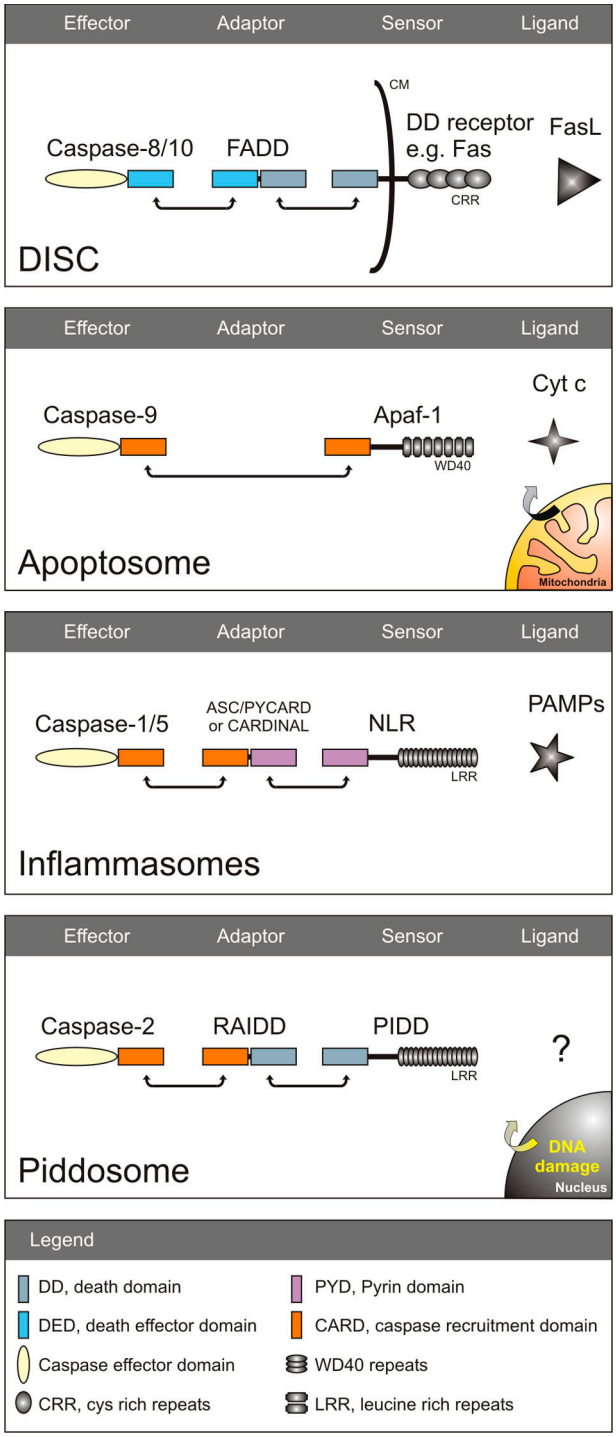

Figure 1. **Overview of the main protein complexes leading to the activation of large prodomain caspases.** Complex formation is initiated by different ligands and sustained by several interaction motifs harbored in complex-residing proteins. The ligand-sensing motifs (e.g., leucine-rich repeats, WD40 repeats, and CRRs) initiate the formation of oligomers. Death domain and death effector domain or CARD–CARD homotypic interactions are crucial for the recruitment and activation of either caspase-8 in the DISC or caspase-9 in the apoptosome, respectively. Caspase-1 or -5 is activated in the different inflammasomes using different adaptors such as ASC/PYCARD or CARDINAL, depending on the type of inflammasome. Several compounds that lead to inflammasome activation, called PAMPs (pathogen-associated molecular patterns), such as bacterial RNA, LPS, or peptidoglycans, were identified. Caspase-2 is activated in the PIDDosome, using the adaptor molecule RAIDD upon DNA damage. How the nuclear damage triggers PIDDosome formation is currently not clear (see also text).

and carcinogenesis (Luo et al., 2005). NF-κB is sequestered in the cytoplasm as an inactive complex bound by a family of inhibitors known as inhibitor of κB (IκB) proteins. In response to a variety of signaling events, the IκB kinase (IKK) complex phosphorylates IκB proteins. This post-translational modification targets IκB for poly-ubiquitination and subsequent degradation by the 26 S proteasome. The degradation of IκB proteins liberates the NF-κB transcription factor, allowing its translocation to the nucleus and activation of its target genes. Many signaling pathways have been elucidated in NF-κB activation, such as those emanating from the TNF receptor family or the IL-1/Toll-like receptor (TLR) family (Bonizzi and Karin, 2004). TLRs are a class of transmembrane cellular pathogen-recognition receptors that survey the extracellular environment and trigger the innate immune response upon interaction with pathogen-associated products.

Prodomain-mediated NF-κB activation by initiator caspases

The activation of NF-κB can be mediated, at least partially, by interaction motifs present in the prodomains of specific caspases (Chaudhary et al., 2000; Kreuz et al., 2004; Lamkanfi et al., 2005). Indeed, several studies have shown that caspases-1, -2, -8, and -10 use similar mechanisms to activate NF-κB, but caspase-9, -11, and -12 do not activate this transcription factor (Chaudhary et al., 2000; Lamkanfi et al., 2004, 2005).

The prodomains of caspases-2, -8, and -10 were shown to be capable of recruiting the NF-κB signaling molecules such as the E3 ubiquitin ligase activity containing factor, tumor necrosis factor-receptor associated factor 2 (TRAF2), the kinase receptor interacting protein 1 (RIP1), and NEMO/IKKγ, eventually leading to increased kinase activity of the IKK complex and NF-κB activation (Chaudhary et al., 2000; Shikama et al., 2003; Lamkanfi et al., 2005) (Fig. 2). Caspase-2 complex formation, initiated by PIDD, has been associated with DNA damage repair (Tinel and Tschopp, 2004). The death domain–containing serine/threonine kinase RIP1 is also recruited in the caspase-2 complex (Lamkanfi et al., 2005), and has been shown to be essential for DNA damage–induced NF-κB activation (Hur et al., 2003). Therefore, the caspase-2 complex, also called PIDDosome (Tinel and Tschopp, 2004), may act as an integrator or molecular switch between inflammatory/anti-apoptotic and apoptotic signaling pathways (Zhivotovsky and Orrenius, 2005). When DNA damage occurs, the PIDDosome complex may emit signals that activate NF-κB, hence allowing the cell to initiate cell survival and DNA repair pathways. Whether caspase-2 has a physiological role in this pathway remains to be established. However, massive DNA damage may also generate too much proteolytically active caspase-2, leading to the continuous activation of apoptotic pathways. Similarly, caspase-8 forms a complex with FLASH (FLICE-associated huge protein) and TRAF2 to initiate the NF-κB activation pathway during TNF signaling in fibroblast and epithelial cell lines (Jun et al., 2005). Although in these cells caspase-8 enzymatic activity is apparently not required for TNF-induced NF-κB activation, antisense-mediated caspase-8 depletion resulted in inhibition of TNF-induced NF-κB activation (Jun et al., 2005),

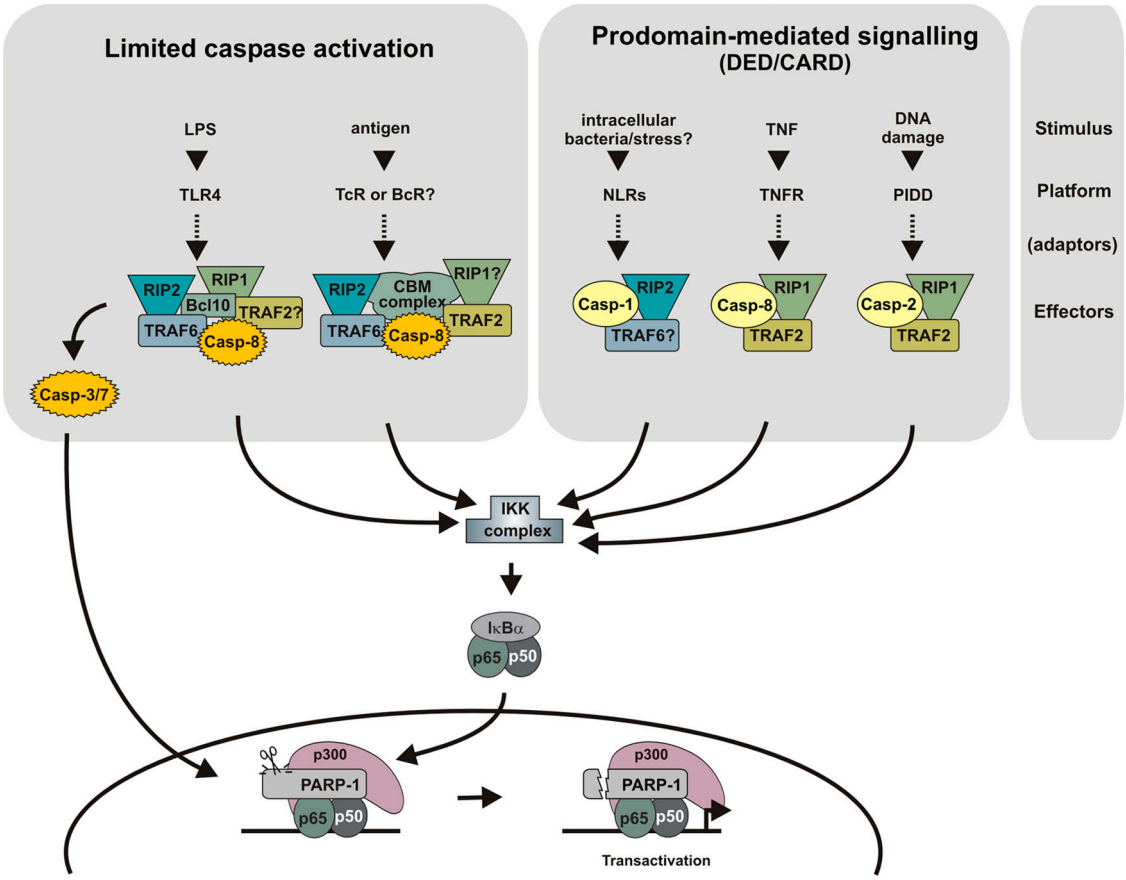

Figure 2. **Proposed model for caspase-mediated NF-κB activation.** Multiple NF-κB signaling pathways can start from protein complexes containing the initiator caspases-1, -2, -8, or -10. These pathways can depend on limited caspase activation resulting in minor enzymatic activity, as in the case of TcR, B-cell receptor, or TLR stimulation, or can start from prodomain-mediated recruitment of NF-κB signaling molecules as in TNF signaling. In addition, cleavage of PARP-1 by caspase-3 or -7 contributes to the transactivation of the NF-κB complex. "Limited caspase activation" is complex-mediated caspase activation that considers only a small fraction of the total pool of a particular caspase and that remains associated with the complex (active caspase depicted by a zigzag line and orange color).

suggesting that caspase-8 can have signaling activities independent of its enzymatic activity.

In addition, Varfolomeev et al. (2005) showed that RNAi-mediated knock-down of caspase-8 abrogates NF-κB activation induced by Apo2L/TRAIL (a member of the TNF family), which would occur when caspase-8, FADD, TRAF-2, RIP-1, and NEMO are recruited to a secondary cytosolic protein complex. The authors propose that caspase-8 enzymatic activity is implicated in Apo2L/TRAIL-dependent NF-κB activation because addition of the pan-caspase inhibitor zVAD-fmk blocks this NF-κB activation. As NF-κB activation upon TRAIL receptor stimulation is observed rather late (hours), as compared with TNF-induced NF-κB activation (minutes), one cannot exclude that the identified signaling complex is formed in response to general apoptotic stress, independently from the TRAIL receptor–bound DISC complex.

In contrast to caspases-2, -8, and -10, the caspase-1 CARD domain is able to recruit the RIP2 kinase, which is involved in TLR- and TcR-mediated NF-κB activation (Kobayashi et al., 2002) (Fig. 2). Experiments using a RIP2 dominant-negative mutant have indicated that RIP2 mediates caspase-1 CARD-induced NF-κB activation (Lamkanfi et al., 2004). Caspase-1 can be recruited to different types of inflammasomes that are initiated upon recognition of intracellular bacteria by NLRs (Martinon and Tschopp, 2004). However, the in vivo relevance of the activation of NF-κB by caspase prodomains remains to be proven.

NF-κB activation initiated by limited activation of initiator caspases

The initial observation that caspase inhibitors efficiently abrogate the proliferation of primary human T cells in vitro and block the production of IL-2 in CD3/CD28-stimulated Jurkat cells suggested a role for caspases beyond the apoptotic context (Kennedy et al., 1999). Several reports have now provided genetic evidence for the role of caspase-8 in the proliferation of immune cells (Chun et al., 2002; Salmena et al., 2003; Beisner et al., 2005; Su et al., 2005). Patients with inactivating mutations in caspase-8 suffer from impaired proliferation of T, B, and NK cells (Chun et al., 2002).

Interestingly, mice in which caspase-8 was conditionally deleted in T cells suffered from similar defects (Salmena et al., 2003). Peripheral T cells from these mice were unable to proliferate after T cell receptor activation. IL-2 production after

CD3/CD28 stimulation decreased significantly in caspase-8–deficient T cells of both human and murine origin (Chun et al., 2002; Salmena et al., 2003). Recently, the circle was closed by demonstrating the essential role of caspase-8 in T cell receptor (TcR)–induced NF-κB activation (Su et al., 2005), confirming its function upstream of IL-2 production and human T cell proliferation. Moreover, through reconstitution experiments in caspase-8–deficient primary human T cells, enzymatically active caspase-8, although unprocessed, was shown to be required for TcR-mediated NF-κB activation (Su et al., 2005). Through labeling of the active center it was estimated that only 10–15% of the total caspase-8 content became enzymatically active after TcR stimulation. In contrast, TNF-induced NF-κB activation in T cells does not require caspase-8 enzymatic activity (Su et al., 2005), similar to observations in fibroblasts and epithelial cells, as discussed above (Jun et al., 2005). However, Salmena et al. (2003) did not observe a difference in TcR-stimulated NF-κB activation in caspase-8–deficient mouse T cells. But these authors looked at NF-κB activation at a rather late time point (6 h), which may explain this apparent contradiction concerning the involvement of caspase-8. In this respect, it has been shown previously that TRAF2 deficiency only delays the kinetics of NF-κB activation in response to TNF, which normally occurs within minutes after treatment (Yeh et al., 1997). At a later time point (90 min), the extent of activated NF-κB in TRAF2-deficient cells was equivalent to that in control cells.

Finally, T, B, and NK cells of patients with caspase-8 deficiency do not show NF-κB activation after stimulation of antigen receptors, Fc receptors, or TLR-4 (Su et al., 2005), confirming the essential role of caspase-8 in NF-κB signaling. What is the mechanism of caspase-8 activation? It was shown that TcR stimulation triggered the recruitment of FADD and caspase-8 to the CARMA-Bcl10-MALT1 (CBM) complex (Fig. 2). Absence of caspase-8 abrogated IKK recruitment and activation by the CBM complex. Although it is clear that enzymatically active caspase-8 is required, the identity of the substrates that need to be processed to initiate the NF-κB signaling cascade remains to be determined. MALT1, also called paracaspase, harbors a caspase-like domain and a death domain, and is required for TcR-induced NF-κB activation (Ruland et al., 2003). In addition to the CBM complex (van Oers and Chen, 2005), TRAF2, TRAF6, and RIP2 were also shown to be essential for TcR-induced NF-κB activation (Kobayashi et al., 2002; Sun et al., 2004). Next to caspase-8, TLR-4 signaling shares several other molecules required for NF-κB activation with the TcR pathway, such as Bcl10 (Xue et al., 2003), RIP1 (Cusson-Hermance et al., 2005), RIP2 (Kobayashi et al., 2002), and TRAF6 (Lomaga et al., 1999).

In contrast to B cells of patients harboring homozygous caspase-8 mutations (Su et al., 2005), the deletion of caspase-8 in murine B cells does not lead to impaired NF-κB activation upon TLR-4 stimulation (Beisner et al., 2005). Nevertheless, caspase-8–deficient B cells fail to proliferate in response to dsRNA or LPS, ligands for TLR-3 and TLR-4, respectively. It remains to be determined whether the observed discrepancy in the response of human and murine caspase-8–deficient B cells is due to a fundamental difference in the signaling pathways involved, to the different nature of caspase-8 deficiency, or to the different experimental designs used to analyze NF-κB activation.

Why would activation of the apoptotic initiator caspase-8 in one cellular context lead to initiation of apoptosis, whereas in another cellular context it activates NF-κB and induces proliferation? This may, at least in part, depend on the level of caspase-8 activation. In proliferating cells, caspase-8 apparently remains unprocessed and would therefore be less active or at least only be active in the complex (Su et al., 2005). In FasL-induced apoptosis, caspase-8 is processed and strongly activated (Peter and Krammer, 2003). One way to modulate the level of caspase-8 activation is by the expression of c-FLIP$_L$, a structural homologue of caspase-8 lacking caspase activity (Tschopp et al., 1998; Micheau et al., 2002). High concentrations of c-FLIP$_L$ prevent full processing and release of active caspase-8 from the DISC, thus blocking the induction of cell death. Mice conditionally lacking c-FLIP in T lymphocytes display severe defects in the development and proliferation of mature T cells (Chau et al., 2005; Zhang and He, 2005), possibly due to enhanced apoptosis sensitivity. However, TcR-induced activation of NF-κB, a caspase-8–dependent phenomenon as discussed above, in cFLIP-deficient thymocytes appears largely intact (Zhang and He, 2005), whereas overexpression of cFLIP in Jurkat T cells increases NF-κB activation upon TcR ligation (Kataoka et al., 2000). Therefore, the role of FLIP proteins in NF-κB activation remains unclear.

Indeed, conflicting data were reported in the literature concerning the role of caspase-8 and cFLIP in TcR-dependent NF-κB activation (Table I). The different levels of cFLIP expression in the different transgenic lines and the various extents of TcR triggering used may explain the apparent discrepancies observed in the literature. Indeed, it was shown that dependent on the anti-CD3 concentration used one can observe increased or reduced T cell proliferation in FLIP transgenic mice (Lens et al., 2002). Taking together the data presented in Table I, we can formulate the following conclusions. Deficiency of either FADD, caspase-8, or cFLIP results in a common phenotype, namely reduced proliferative response upon TcR stimulation, demonstrating that these proteins act in a common pathway starting from the TcR. Although it has been extensively documented that NF-κB is required for T cell proliferation and activation (Li and Verma, 2002), the data discussed above suggest that TcR-induced proliferation does not solely depend on NF-κB activation. Viral FLIPs are proteins that resemble the prodomain of caspase-8 and inhibit the recruitment of caspase-8 to the DISC (Ekert et al., 1999). Human herpesvirus 8 (HHV-8) vFLIP can also activate the NF-κB pathway, a property not shared by other vFLIPs (Chaudhary et al., 1999). This observation reminds us of the similar situation at the level of CARD-containing caspases—some are capable of inducing NF-κB activation, whereas others are not (Lamkanfi et al., 2004, 2005). Transgenic overexpression of HHV-8 vFLIP in the lymphoid compartment leads to constitutive NF-κB activation and increased incidence of lymphomas, but has no significant effect on Fas-induced apoptosis or the development and maturation of lymphocytes. Therefore, one can fairly conclude that FADD, caspase-8, and cFLIP are molecularly linked, at least in normal

Table I. **Comparison of phenotypes related to TcR-induced proliferation and NF-κB activation in different human and mouse models**

Protein	Phenotype	Reference
FADD	**Deficient**	
	FADD is required for TcR-induced proliferation in murine T cells	(Zhang et al., 1998; Zhang et al., 2005)
	Transgene	
	Dominant-negative FADD (FADD-DN)-expressing murine T cells display proliferative defects following TcR activation	(Newton et al., 1998; Arechiga et al., 2005)
	FADD-DN does not block TcR-mediated NF-κB activation	(Arechiga et al., 2005)
Caspase-8	**Deficient**	
	Caspase-8–deficient mouse T cells show defective TcR-induced proliferation, but NF-κB activation not affected	(Salmena et al., 2003)
	Caspase-8–deficient human T cells show hampered NF-κB activation upon TcR stimulation. Caspase-8 enzymatic activity required for TcR-induced NF-κB.	(Su et al., 2005)
cFLIP	**Deficient**	
	$cFLIP^{-/-}$ mouse T cells are impaired in proliferation in response to TcR stimulation	(Chau et al., 2005; Zhang and He, 2005)
	TcR-induced NF-κB activation in $cFLIP^{-/-}$ thymocytes appears intact	(Zhang and He, 2005)
	Transgene	
	cFLIP transgene in T cell compartment shows spontaneous loss of IkBα, which may reflect activation of NF-κB	(Kataoka et al., 2000)
	Suboptimal doses of TcR stimulation reveal increased proliferation in cFLIP transgenic T cells	(Lens et al., 2002)
	Increased NF-κB activation upon TcR triggering of cFLIP transgenic T cells	(Dohryman et al., 2005)
	Suppressed TcR-induced proliferation in cFLIP transgenic T cells	(Tai et al., 2004)

T cell development and functioning. The exact role of these molecules in the NF-κB activation pathway remains to be determined. In this respect, it would be interesting to analyze the NF-κB signaling pathways in transgenic mice overexpressing the caspase-8 inhibitor CrmA (cytokine response modifier A) (Smith et al., 1996). What really determines the final decision between FADD/caspase-8–mediated apoptosis or FADD/caspase-8–mediated proliferation is not clear today, but availability and specific proteolytsis of particular substrates will certainly contribute.

NF-κB activation initiated by limited activation of executioner caspases

Recently, a possible involvement of executioner caspases in NF-κB activation was suggested. Although cleavage of poly(ADP-ribose)polymerase-1 (PARP-1) at the DEVD site has been used as a canonical hallmark of caspase-3/-7 activation and apoptosis, the in vivo significance of this cleavage was largely unknown. Initially, it was thought that PARP-1 cleavage in apoptotic cells represented a way to inactivate the DNA repair capacity. Recent genetic evidence suggests a novel role for caspase-generated fragments of PARP-1 in inflammatory responses. Indeed, LPS-stimulated macrophages from knock-in mice expressing caspase-resistant PARP-1 display impaired NF-κB–mediated gene activation despite normal binding of NF-κB to DNA (Petrilli et al., 2004).

As discussed above, caspase-8 is required for nuclear translocation of NF-κB (Su et al., 2005), whereas executioner caspases apparently act at the NF-κB transactivation level following DNA binding through a PARP-1–mediated mechanism (Petrilli et al., 2004) (Fig. 2). Indeed, it had previously been shown that PARP-1 can interact with both subunits of NF-κB (p50 and p65) and with the transcriptional coactivator p300 (Hassa et al., 2001, 2003). Whether the enzymatic activity of PARP-1 is required for the enhanced transcriptional activity of NF-κB is a matter of dispute. Some reports show that enzymatic activity is not required for coactivation of NF-κB by p300 in TNF- or LPS-stimulated primary fibroblasts or macrophages (Hassa et al., 2003; Petrilli et al., 2004). However, other studies using PARP-1 inhibitors do indicate a role for PARP-1 enzymatic activity in the enhancement of NF-κB transcriptional activity (Chiarugi and Moskowitz, 2003; Nakajima et al., 2004). The strong phenotype of the caspase-resistant PARP-1 knock-in mice, such as their resistance to endotoxic shock and to intestinal and renal ischemia-reperfusion, further support an in vivo contribution of executioner caspases to inflammatory responses through PARP-1 cleavage. Hence, caspase-mediated PARP-1 cleavage under nonapoptotic conditions could contribute to the level of NF-κB transcriptional activity.

The possible role of executioner caspases in the activation of NF-κB may at least partially explain the multitude of nonapoptotic functions that have recently been attributed to this caspase subfamily. For example, caspase-3 was reported to be active in the nuclei of dividing cells in the proliferative regions of the rat forebrain (Yan et al., 2001). Furthermore, caspase-dependent cleavage of $p27^{KIP1}$ occurs in proliferating lymphoid cells (Frost et al., 2001), and B cells from caspase-3–deficient mice can hyperproliferate (Woo et al., 2003). The latter finding indicates that caspase-3 may function as a negative regulator of B cell cycling. In this respect, caspase-3 was shown to be required for the cleavage of the Cdk inhibitor p21 in B cells (Woo et al., 2003). Similar studies in conditional knock-out mice may reveal additional roles for caspases in cell cycle regulation.

Conclusions and perspectives

In summary, two main mechanisms of caspase-mediated NF-κB activation can be distinguished. The prodomains of the initiator caspases-1, -2, and -8 can engage in NF-κB signaling

through the recruitment of NF-κB–signaling molecules such as TRAF-2/RIP-1 or TRAF-6/RIP-2. However, we are aware that most of these data are still based on overexpression studies and the physiological relevance of these pathways should be further addressed. On the other hand, limited levels of caspase activation and substrate cleavage allow both initiator and executioner caspases to operate in nonapoptotic functions such as NF-κB activation. The exact caspase-dependent signaling pathways to gene activation remain to be determined. Regardless of the signaling mechanism involved, NF-κB activation can prevent the induction of apoptosis by up-regulating anti-apoptotic mechanisms such as anti-apoptotic Bcl-2 family members (Wang et al., 1999), inhibitor of apoptosis proteins (IAPs) (Weber and Menko, 2005), and $FLIP_L$ (Micheau et al., 2002). This is in marked contrast to conditions of full-blown proteolytic activation of caspases observed during apoptosis. The concomitant proteolysis of apoptosis-associated substrates results in the release of pro-apoptotic mitochondrial factors in the cytosol, thus strongly enhancing the apoptotic outcome. Altogether, the involvement of caspases in nonapoptotic functions suggests that therapeutic inhibition of caspase activity to prevent cell death may have broader implications than initially conceived.

We apologize to the authors that made contributions to the field, but have not been cited due to space limitations. We thank Amin Bredan for editorial help.

This work was supported in part by the Interuniversitaire Attractiepolen V (IUAP-P5/12-120C1402), the Belgian Federation Against Cancer, the Fonds voor Wetenschappelijk Onderzoek-Vlaanderen (grant 3G.0006.01 and G.0133.05), EC-RTD grants (QLG1-CT-1999-00739 and LSHB-CT-2005-019067), a UGent-cofinancing EU project (011C0300), and GOA project (12050502). T. Vanden Berghe was supported by a grant from IUAP-P5/12-120C1402 and has now a postdoctoral grant from the Ghent University (06524-BOF).

Submitted: 15 September 2005
Accepted: 20 March 2006

References

Arechiga, A.F., B.D. Bell, J.C. Solomon, I.H. Chu, C.L. Dubois, B.E. Hall, T.C. George, D.M. Coder, and C.M. Walsh. 2005. Cutting edge: FADD is not required for antigen receptor-mediated NF-kappaB activation. *J. Immunol.* 175:7800–7804.

Beisner, D.R., I.L. Ch'en, R.V. Kolla, A. Hoffmann, and S.M. Hedrick. 2005. Cutting edge: innate immunity conferred by B cells is regulated by caspase-8. *J. Immunol.* 175:3469–3473.

Boatright, K.M., and G.S. Salvesen. 2003. Mechanisms of caspase activation. *Curr. Opin. Cell Biol.* 15:725–731.

Bonizzi, G., and M. Karin. 2004. The two NF-kappaB activation pathways and their role in innate and adaptive immunity. *Trends Immunol.* 25:280–288.

Cain, K., S.B. Bratton, and G.M. Cohen. 2002. The Apaf-1 apoptosome: a large caspase-activating complex. *Biochimie.* 84:203–214.

Chau, H., V. Wong, N.J. Chen, H.L. Huang, W.J. Lin, C. Mirtsos, A.R. Elford, M. Bonnard, A. Wakeham, A.I. You-Ten, et al. 2005. Cellular FLICE-inhibitory protein is required for T cell survival and cycling. *J. Exp. Med.* 202:405–413.

Chaudhary, P.M., A. Jasmin, M.T. Eby, and L. Hood. 1999. Modulation of the NF-kappa B pathway by virally encoded death effector domains-containing proteins. *Oncogene.* 18:5738–5746.

Chaudhary, P.M., M.T. Eby, A. Jasmin, A. Kumar, L. Liu, and L. Hood. 2000. Activation of the NF-kappaB pathway by caspase 8 and its homologs. *Oncogene.* 19:4451–4460.

Chiarugi, A., and M.A. Moskowitz. 2003. Poly(ADP-ribose) polymerase-1 activity promotes NF-kappaB-driven transcription and microglial activation: implication for neurodegenerative disorders. *J. Neurochem.* 85:306–317.

Chun, H.J., L. Zheng, M. Ahmad, J. Wang, C.K. Speirs, R.M. Siegel, J.K. Dale, J. Puck, J. Davis, C.G. Hall, et al. 2002. Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. *Nature.* 419:395–399.

Cusson-Hermance, N., S. Khurana, T.H. Lee, K.A. Fitzgerald, and M.A. Kelliher. 2005. Rip1 mediates the Trif-dependent toll-like receptor 3- and 4-induced NF-{kappa}B activation but does not contribute to interferon regulatory factor 3 activation. *J. Biol. Chem.* 280:36560–36566.

Dohrman, A., T. Kataoka, S. Cuenin, J.Q. Russell, J. Tschopp, and R.C. Budd. 2005. Cellular FLIP (long form) regulates CD8+ T cell activation through caspase-8-dependent NF-kappa B activation. *J. Immunol.* 174:5270–5278.

Ekert, P.G., J. Silke, and D.L. Vaux. 1999. Caspase inhibitors. *Cell Death Differ.* 6:1081–1086.

Frost, V., S. Delikat, S. Al-Mehairi, and A.J. Sinclair. 2001. Regulation of p27KIP1 in Epstein-Barr virus-immortalized lymphoblastoid cell lines involves non-apoptotic caspase cleavage. *J. Gen. Virol.* 82:3057–3066.

Ghayur, T., S. Banerjee, M. Hugunin, D. Butler, L. Herzog, A. Carter, L. Quintal, L. Sekut, R. Talanian, M. Paskind, et al. 1997. Caspase-1 processes IFN-gamma-inducing factor and regulates LPS-induced IFN-gamma production. *Nature.* 386:619–623.

Hassa, P.O., M. Covic, S. Hasan, R. Imhof, and M.O. Hottiger. 2001. The enzymatic and DNA binding activity of PARP-1 are not required for NF-kappa B coactivator function. *J. Biol. Chem.* 276:45588–45597.

Hassa, P.O., C. Buerki, C. Lombardi, R. Imhof, and M.O. Hottiger. 2003. Transcriptional coactivation of nuclear factor-kappaB-dependent gene expression by p300 is regulated by poly(ADP)-ribose polymerase-1. *J. Biol. Chem.* 278:45145–45153.

Hur, G.M., J. Lewis, Q. Yang, Y. Lin, H. Nakano, S. Nedospasov, and Z.G. Liu. 2003. The death domain kinase RIP has an essential role in DNA damage-induced NF-kappa B activation. *Genes Dev.* 17:873–882.

Jun, J.I., C.W. Chung, H.J. Lee, J.O. Pyo, K.N. Lee, N.S. Kim, Y.S. Kim, H.S. Yoo, T.H. Lee, E. Kim, and Y.K. Jung. 2005. Role of FLASH in caspase-8-mediated activation of NF-kappaB: dominant-negative function of FLASH mutant in NF-kappaB signaling pathway. *Oncogene.* 24:688–696.

Kataoka, T., R.C. Budd, N. Holler, M. Thome, F. Martinon, M. Irmler, K. Burns, M. Hahne, N. Kennedy, M. Kovacsovics, and J. Tschopp. 2000. The caspase-8 inhibitor FLIP promotes activation of NF-kappaB and Erk signaling pathways. *Curr. Biol.* 10:640–648.

Kennedy, N.J., T. Kataoka, J. Tschopp, and R.C. Budd. 1999. Caspase activation is required for T cell proliferation. *J. Exp. Med.* 190:1891–1896.

Kobayashi, K., N. Inohara, L.D. Hernandez, J.E. Galan, G. Nunez, C.A. Janeway, R. Medzhitov, and R.A. Flavell. 2002. RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. *Nature.* 416:194–199.

Kreuz, S., D. Siegmund, J.J. Rumpf, D. Samel, M. Leverkus, O. Janssen, G. Hacker, O. Dittrich-Breiholz, M. Kracht, P. Scheurich, and H. Wajant. 2004. NFkappaB activation by Fas is mediated through FADD, caspase-8, and RIP and is inhibited by FLIP. *J. Cell Biol.* 166:369–380.

Lamkanfi, M., W. Declercq, B. Depuydt, M. Kalai, X. Saelens, and P. Vandenabeele. 2003. The caspase family. *In* Caspases—Their Role in Cell Death and Cell Survival. M. Los and H. Walczak, editors. Landes Bioscience and Kluwer Academic, New York. 1–40.

Lamkanfi, M., M. Kalai, X. Saelens, W. Declercq, and P. Vandenabeele. 2004. Caspase-1 activates NF-kappa B independent of its enzymatic activity. *J. Biol. Chem.* 279:24785–24793.

Lamkanfi, M., K. D'Hondt, L. Vande Walle, M. van Gurp, G. Denecker, J. Demeulemeester, M. Kalai, W. Declercq, X. Saelens, and P. Vandenabeele. 2005. A novel caspase-2 complex containing TRAF2 and RIP1. *J. Biol. Chem.* 280:6923–6932.

Lens, S.M., T. Kataoka, K.A. Fortner, A. Tinel, I. Ferrero, R.H. MacDonald, M. Hahne, F. Beermann, A. Attinger, H.A. Orbea, et al. 2002. The caspase 8 inhibitor c-FLIP(L) modulates T-cell receptor-induced proliferation but not activation-induced cell death of lymphocytes. *Mol. Cell. Biol.* 22:5419–5433.

Li, P., H. Allen, S. Banerjee, S. Franklin, L. Herzog, C. Johnston, J. McDowell, M. Paskind, L. Rodman, J. Salfeld, et al. 1995. Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. *Cell.* 80:401–411.

Li, Q., and I.M. Verma. 2002. NF-kappaB regulation in the immune system. *Nat. Rev. Immunol.* 2:725–734.

Lomaga, M.A., W.C. Yeh, I. Sarosi, G.S. Duncan, C. Furlonger, A. Ho, S. Morony, C. Capparelli, G. Van, S. Kaufman, et al. 1999. TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev.* 13:1015–1024.

Luo, J.L., H. Kamata, and M. Karin. 2005. IKK/NF-kappaB signaling: balancing life and death–a new approach to cancer therapy. *J. Clin. Invest.* 115:2625–2632.

Martinon, F., and J. Tschopp. 2004. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell.* 117:561–574.

Martinon, F., and J. Tschopp. 2005. NLRs join TLRs as innate sensors of pathogens. *Trends Immunol.* 26:447–454.

Micheau, O., M. Thome, P. Schneider, N. Holler, J. Tschopp, D.W. Nicholson, C. Briand, and M.G. Grutter. 2002. The long form of FLIP is an activator of caspase-8 at the Fas death-inducing signaling complex. *J. Biol. Chem.* 277:45162–45171.

Nakajima, H., H. Nagaso, N. Kakui, M. Ishikawa, T. Hiranuma, and S. Hoshiko. 2004. Critical role of the automodification of poly(ADP-ribose) polymerase-1 in nuclear factor-kappaB-dependent gene expression in primary cultured mouse glial cells. *J. Biol. Chem.* 279:42774–42786.

Newton, K., A.W. Harris, M.L. Bath, K.G. Smith, and A. Strasser. 1998. A dominant interfering mutant of FADD/MORT1 enhances deletion of autoreactive thymocytes and inhibits proliferation of mature T lymphocytes. *EMBO J.* 17:706–718.

Peter, M.E., and P.H. Krammer. 2003. The CD95(APO-1/Fas) DISC and beyond. *Cell Death Differ.* 10:26–35.

Petrilli, V., Z. Herceg, P.O. Hassa, N.S. Patel, R. Di Paola, U. Cortes, L. Dugo, H.M. Filipe, C. Thiemermann, M.O. Hottiger, et al. 2004. Noncleavable poly(ADP-ribose) polymerase-1 regulates the inflammation response in mice. *J. Clin. Invest.* 114:1072–1081.

Ruland, J., G.S. Duncan, A. Wakeham, and T.W. Mak. 2003. Differential requirement for Malt1 in T and B cell antigen receptor signaling. *Immunity.* 19:749–758.

Salmena, L., B. Lemmers, A. Hakem, E. Matysiak-Zablocki, K. Murakami, P.Y. Au, D.M. Berry, L. Tamblyn, A. Shehabeldin, E. Migon, et al. 2003. Essential role for caspase 8 in T-cell homeostasis and T-cell-mediated immunity. *Genes Dev.* 17:883–895.

Shikama, Y., M. Yamada, and T. Miyashita. 2003. Caspase-8 and caspase-10 activate NF-kappaB through RIP, NIK and IKKalpha kinases. *Eur. J. Immunol.* 33:1998–2006.

Smith, K.G., A. Strasser, and D.L. Vaux. 1996. CrmA expression in T lymphocytes of transgenic mice inhibits CD95 (Fas/APO-1)-transduced apoptosis, but does not cause lymphadenopathy or autoimmune disease. *EMBO J.* 15:5167–5176.

Su, H., N. Bidere, L. Zheng, A. Cubre, K. Sakai, J. Dale, L. Salmena, R. Hakem, S. Straus, and M. Lenardo. 2005. Requirement for caspase-8 in NF-kappaB activation by antigen receptor. *Science.* 307:1465–1468.

Sun, L., L. Deng, C.K. Ea, Z.P. Xia, and Z.J. Chen. 2004. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol. Cell.* 14:289–301.

Tai, T.S., L.W. Fang, and M.Z. Lai. 2004. c-FLICE inhibitory protein expression inhibits T-cell activation. *Cell Death Differ.* 11:69–79.

Tinel, A., and J. Tschopp. 2004. The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. *Science.* 304:843–846.

Tschopp, J., M. Irmler, and M. Thome. 1998. Inhibition of fas death signals by FLIPs. *Curr. Opin. Immunol.* 10:552–558.

van Oers, N.S., and Z.J. Chen. 2005. Cell biology. Kinasing and clipping down the NF-kappa B trail. *Science.* 308:65–66.

Varfolomeev, E., H. Maecker, D. Sharp, D. Lawrence, M. Renz, D. Vucic, and A. Ashkenazi. 2005. Molecular determinants of kinase pathway activation by Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand. *J. Biol. Chem.* 280:40599–40608.

Wang, C.Y., D.C. Guttridge, M.W. Mayo, and A.S. Baldwin Jr. 1999. NF-kappaB induces expression of the Bcl-2 homologue A1/Bfl-1 to preferentially suppress chemotherapy-induced apoptosis. *Mol. Cell. Biol.* 19:5923–5929.

Wang, S., M. Miura, Y.K. Jung, H. Zhu, E. Li, and J. Yuan. 1998. Murine caspase-11, an ICE-interacting protease, is essential for the activation of ICE. *Cell.* 92:501–509.

Weber, G.F., and A.S. Menko. 2005. The canonical intrinsic mitochondrial death pathway has a non-apoptotic role in signaling lens cell differentiation. *J. Biol. Chem.* 280:22135–22145.

Woo, M., R. Hakem, C. Furlonger, A. Hakem, G.S. Duncan, T. Sasaki, D. Bouchard, L. Lu, G.E. Wu, C.J. Paige, and T.W. Mak. 2003. Caspase-3 regulates cell cycle in B cells: a consequence of substrate specificity. *Nat. Immunol.* 4:1016–1022.

Xue, L., S.W. Morris, C. Orihuela, E. Tuomanen, X. Cui, R. Wen, and D. Wang. 2003. Defective development and function of Bcl10-deficient follicular, marginal zone and B1 B cells. *Nat. Immunol.* 4:857–865.

Yan, X.X., J. Najbauer, C.C. Woo, K. Dashtipour, C.E. Ribak, and M. Leon. 2001. Expression of active caspase-3 in mitotic and postmitotic cells of the rat forebrain. *J. Comp. Neurol.* 433:4–22.

Yeh, W.C., A. Shahinian, D. Speiser, J. Kraunus, F. Billia, A. Wakeham, J.L. de la Pompa, D. Ferrick, B. Hum, N. Iscove, et al. 1997. Early lethality, functional NF-kappaB activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity.* 7:715–725.

Zhang, J., D. Cado, A. Chen, N.H. Kabra, and A. Winoto. 1998. Fas-mediated apoptosis and activation-induced T-cell proliferation are defective in mice lacking FADD/Mort1. *Nature.* 392:296–300.

Zhang, N., and Y.W. He. 2005. An essential role for c-FLIP in the efficient development of mature T lymphocytes. *J. Exp. Med.* 202:395–404.

Zhang, Y., S. Rosenberg, H. Wang, H.Z. Imtiyaz, Y.J. Hou, and J. Zhang. 2005. Conditional Fas-associated death domain protein (FADD):GFP knockout mice reveal FADD is dispensable in thymic development but essential in peripheral T cell homeostasis. *J. Immunol.* 175:3033–3044.

Zhivotovsky, B., and S. Orrenius. 2005. Caspase-2 function in response to DNA damage. *Biochem. Biophys. Res. Commun.* 331:859–867.