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REVIEW

Regulation of NF-κB signaling by caspases and MALT1 paracaspase

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Caspases are intracellular proteases that are best known for their function in apoptosis signaling. It has become evident that many caspases also function in other signaling pathways that propagate cell proliferation and inflammation, but studies on the inflammatory function of caspases have mainly been limited to caspase-1-mediated cytokine processing. Emerging evidence, however, indicates an important contribution of caspases as mediators or regulators of nuclear factor-κB (NF-κB) signaling, which plays a key role in inflammation and immunity. Much still needs to be learned about the mechanisms that govern the activation and regulation of NF-κB by caspases, and this review provides an update of this area. Whereas apoptosis signaling is dependent on the catalytic activity of caspases, they mainly act as scaffolding platforms for other signaling proteins in the case of NF-κB signaling. Caspase proteolytic activity, however, counteracts the pro-survival function of NF-κB by cleaving specific signaling molecules. A striking exception is the paracaspase mucosa-associated lymphoid tissue 1 (MALT1), whose adaptor and proteolytic activity are both needed to initiate a full blown NF-κB response in antigen-stimulated lymphocytes. Understanding the role of caspases and MALT1 in the regulation of NF-κB signaling is of high interest for therapeutic immunomodulation.

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Introduction

Signal transduction generally involves ion fluxes, generation of secondary metabolites, protein-protein interactions and a series of post-translational protein modifications [1]. Many of these modifications, such as phosphorylation and ubiquitination, are reversible. In contrast, proteolytic cleavage as a signaling event causes an irreversible change in the target protein. A unique property of protein cleavage as a signaling event is its ability to separate two functional domains of a protein, which can result in novel properties either through a change in localization of one or both the fragments [2] or through alteration of inter- or intra-molecular interactions [3]. The functional consequences of protein cleavage are mostly transient due to the dynamic exchange of uncleaved and cleaved proteins in the signaling complex and protein turnover in general. Several proteases control key steps in immune cell signaling and represent interesting targets for therapeutic immunomodulation [4, 5]. In this context, caspases are a family of cysteine proteases that have attracted a lot of attention. The caspase family is best known for its function in apoptosis signaling or in the processing of the pro-inflammatory cytokines interleukin (IL)-1β and IL-18 [6]. However, evidence has emerged over the past few years that a number of caspases thought to be involved solely in apoptosis or cytokine maturation also employ other ways to participate in specific aspects of immunity [7, 8]. For example, and as discussed here, several caspases participate in the regulation of inflammatory gene expression by mediating or modulating nuclear factor-κB (NF-κB) signaling.

NF-κB is a transcription factor that plays a central role in inflammation and immunity by regulating the expression of many target genes that mediate distinct events in the inflammatory response. The NF-κB system is a paradigm for signaling in response to inflammatory stimuli. Given the large amount of evidence that dysregulated NF-κB signaling is involved in the onset of autoimmune

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diseases and different types of cancer, there is a lot of interest in this pathway for drug targeting [9]. In this review, we discuss recent insights into this specific signal transduction cascade, and the way it is regulated by caspases. In addition, we discuss its regulation by mucosa-associated lymphoid tissue 1 (MALT1) paracaspase, which is structurally related to caspases and whose proteolytic activity has only recently been identified.

NF-κB signaling: multiple convergent pathways

The NF-κB pathway is critically involved in many aspects of inflammation and immunity, from regulation of lymphoid organ development to the functioning of innate and adaptive cells. Moreover, NF-κB is a key regulator of cell survival and plays a role in the development of cancer. In mammals, the NF-κB transcription factor family consists of five members: p65 (also termed RelA), RelB, c-Rel, p50 and p52 (p50 and p52 being generated by processing of the precursor molecules p105 and p100, respectively). They can form various homodimeric or heterodimeric complexes, with the p50/p65 heterodimer being characterized in most detail. NF-κB dimers are sequestered in the cytoplasm by binding to specific IκB (inhibitor of NF-κB) proteins [10], of which IκBα is the best studied member and displays all defining characteristics of an NF-κB inhibitor. IκBα is primarily associated with the p50/p65 NF-κB dimer, and stimulus-induced phosphorylation of IκBα results in its K48-linked polyubiquitination and proteasomal degradation, allowing NF-κB nuclear translocation and DNA binding [11, 12]. NF-κB activation is controlled by two IκB kinases (IKK), IKKα and IKKβ, which form a complex with the regulatory subunit IKKγ (also known as NEMO). In the canonical NF-κB pathway, which is activated by inflammatory cytokines such as tumor necrosis factor (TNF), antigen receptors (T or B cell receptors – TCR/BCR) and pathogen- or danger-associated molecules (PAMPs or DAMPs, respectively; Figure 1), IKKβ is both necessary and sufficient to phosphorylate IκBα in an IKKγ-dependent manner [13-18]. Activation of IKKβ is dependent on its phosphorylation, but whether this occurs via auto-phosphorylation or phosphorylation by another kinase remains a matter of debate. In this context, transforming growth factor-β-activated kinase 1 (TAK1) and mitogen-activated protein kinase (MAPK) kinase kinase 3 (MEKK3) play non-redundant roles in IKK activation [19-22]. In contrast, the non-canonical NF-κB pathway that is induced by specific members of the TNF cytokine family, such as B cell-activating factor belonging to the *TNF* family (BAFF), lymphotoxin-β and CD40 ligand, relies on IKKα and is independent of IKKβ and IKKγ. IKKα is believed to specifically phosphorylate p100 associated with RelB, leading to its proteasomal processing to p52 [23]. Also in this case, the upstream signaling events remain poorly understood, although stabilization of NIK (NF-κB-inducing kinase), which mediates phosphorylation of IKKα, is a critical step [23, 24]. Since caspases have so far not been implicated in this pathway, we will not discuss it further in this review. A third NF-κB activating pathway is initiated as part of the DNA damage response and represents a cell survival mechanism [25]. Upon genotoxic stress, p53-induced protein with a death domain (PIDD) translocates to the nucleus and forms a complex with receptor interacting protein 1 (RIP1) and NEMO. A second, parallel pathway triggered upon DNA damage leads to the phosphorylation and activation of the ataxia telangiectasia mutated (ATM) kinase. The two pathways converge when NEMO is phosphorylated and ubiquitinated in an ATM-dependent manner and translocates back to the cytosol, where it can activate the IKK complex. When the damage is too severe, apoptosis is initiated by an alternative complex containing PIDD, RIP-associated ICH-1/CED-3 homologous protein with a death domain (RAIDD) and caspase-2 [26].

Canonical NF-κB signaling initiated by different receptors requires the formation of proximal protein-protein interactions that are receptor-specific, but ultimately converge in the activation of the IKK complex. Most of our knowledge on this pathway comes from studies of TNF receptor 1 signaling. TNF stimulation results in the recruitment of TNF receptor 1-associated death domain protein (TRADD) and RIP1, which function as adaptor proteins for the E3 ubiquitin ligases TNF receptor-associated factor (TRAF) 2 and TRAF5, which in turn bind the E3 ubiquitin ligases cellular inhibitor of apoptosis (cIAP) 1 and cIAP2. On TNF stimulation, TNF-receptor bound RIP1 is rapidly modified by K63-linked polyubiquitin chains. TRAF2/5 and cIAP1/2 are good candidates for RIP1 ubiquitination, but the specific role of each is still unclear. The polyubiquitin chains on RIP1 are believed to create a scaffold to recruit the IKK and TAK1 complex via the ubiquitin-binding proteins NEMO and TAB1/2, respectively. TRADD also recruits Fas-associated protein with death domain (FADD), which together with RIP1 forms a platform for caspase-8 to initiate apoptosis signaling. It was recently demonstrated that RIP1 ubiquitination and NEMO binding to RIP1 prevents the latter from engaging caspase-8 and initiating apoptosis [27], illustrating that RIP1 ubiquitination can determine whether RIP1 functions as a pro-survival or pro-cell death molecule. The recent identification of a distinct E2/E3 enzyme complex that modifies NEMO with linear polyubiquitin chains and is essential for TNF-

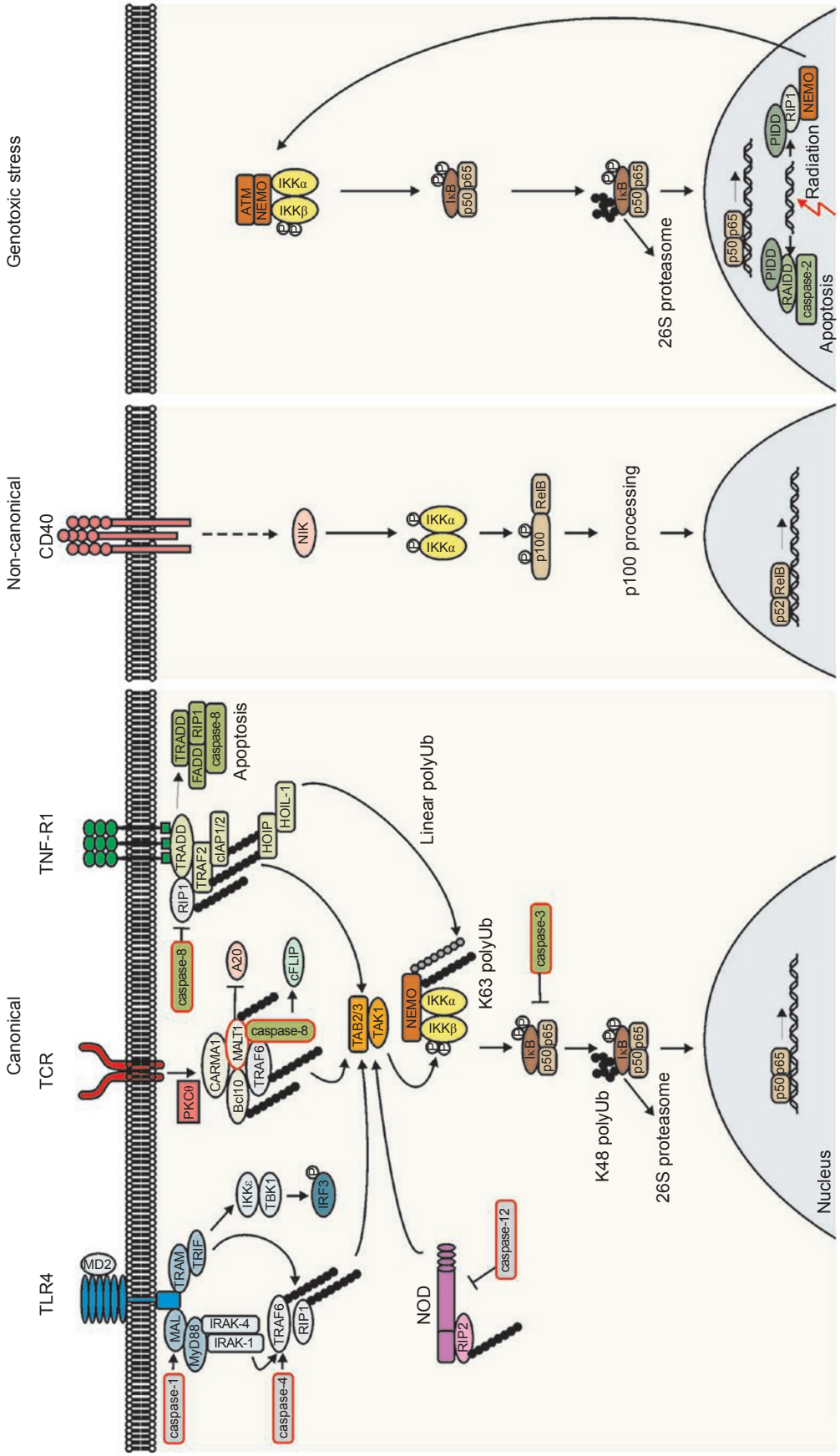

Figure 1 NF-κB signaling pathways initiated by TLR4, TCR, TNF receptor 1, CD40 and genotoxic stress. Left: canonical NF-κB signaling is initiated by the inducible formation of multiprotein complexes that can undergo non-degradative polyubiquitination (K63-linked or linear) and which are recognized by TAK1 and IKK kinase complexes. This leads to the activation of IKKβ, which phosphorylates the NF-κB inhibitor protein IκBα, resulting in its proteasomal degradation and nuclear translocation of NF-κB (depicted as p50/p65). TNF receptor 1 can also initiate the formation of a caspase-8-containing complex that signals apoptosis. The places where caspases have been proposed to regulate NF-κB signaling are indicated. Middle: some receptors initiate a non-canonical NF-κB signaling pathway that results in the activation of IKKα, which phosphorylates the NF-κB inhibitor p100, resulting in its processing to p52 and the formation of p52/RelB NF-κB complexes. Receptors initiating this pathway also initiate the formation of multiprotein complexes and ubiquitination (not shown in the figure), which results in the activation of the kinase NIK, which activates IKKα. Right: NF-κB activation by genotoxic stress results in the activation of a multiprotein complex (PIDDosome) in which the IKK adaptor NEMO is recruited. This is the beginning of a sequential series of modifications on NEMO, which alter its subcellular localization, providing a means to link genotoxic stress to the cytosolic IKK complex. An alternative PIDDosome complex recruits caspase-2 and initiates apoptosis.

activated NF-κB signaling adds further complexity [28]. Members of the Toll-like receptor (TLR)/IL-1 receptor (IL-1R) family are also potent activators of the canonical NF-κB pathway. For example, lipopolysaccharide (LPS) induces the recruitment of Toll/IL-1 receptor adaptor protein (TIRAP), also referred to as Mal, and TRAM, which most likely serve as bridging factors to recruit myeloid differentiation primary response gene 88 (MyD88) and TIR domain-containing adaptor inducing interferon-β (TRIF), respectively. MyD88 in turn recruits members of the IL-1R-associated kinase (IRAK) family and TRAF6, leading to oligomerization and self-ubiquitination of TRAF6 [29]. TRIF also recruits TRAF6 [30] and RIP1 [31] via a direct interaction. Both pathways then activate TAK1 and IKK in a ubiquitination-dependent manner similar to the TNF pathway. TRIF can also bind TRAF3 and activate TANK-binding kinase 1 (TBK1)/IKKε [30, 32], resulting in the phosphorylation of interferon (IFN) regulator factor (IRF) 3 and IRF7, which regulate the production of type I IFN. NF-κB signaling can also be initiated by members of the intracellular NOD-like receptor (NLR) family. NOD1 and NOD2 activation by peptides derived from bacterial peptidoglycans results in the binding of RIP2, which signals towards NF-κB [33, 34], most likely by engaging members of the TRAF family [35]. As a final example, activation of NF-κB by the TCR involves the protein kinase C (PKC) θ-mediated phosphorylation of the caspase activation and recruitment domain (CARD)-containing protein CARMA1, resulting in the recruitment of Bcl10 and MALT1, thus leading to the formation of a stable CBM complex [36]. Via a poorly defined mechanism, the latter then recruits TRAF2 and TRAF6, resulting in the ubiquitination-dependent activation of TAK1 and IKK as described above. The exact role of protein anchored polyubiquitin chains remains unclear, as it was recently suggested that unanchored polyubiquitin chains can directly activate the TAK1 complex [37].

"Apoptotic" and "inflammatory" caspases – a classification with exceptions

Caspases are cysteine-dependent aspartate-specific proteases, which can mediate limited, specific cleavage of key cellular components to trigger certain signaling cascades, of which caspase-mediated apoptosis signaling is best known. In humans, 12 caspases have been identified, namely caspase-1 to -10, -12 and -14 [38]. They are all widely expressed, except for caspase-14, which is mainly restricted to barrier-forming tissues [39]. Caspases are synthesized as single-chain proteins, with an N-terminal prodomain preceding the conserved catalytic domain. Caspase activation is often followed by their proteolytic maturation, which involves the removal of the prodomain and/or cleavage of the catalytic domain with the formation of a large (p20) and small (p10) subunit. The large subunit contains the catalytic dyad residues Cys and His, while the small subunit contains several residues that form the substrate-binding pocket. The active caspase form is a dimer of p20p10/p10p20 symmetry and has two active sites [38]. On the basis of their known major functions, caspases are grouped into apoptotic (caspase-2, -3, -6, -7, -8, -9 and -10) and non-apoptotic/pro-inflammatory (caspase-1, -4, -5 and -12) caspases. Within the apoptotic subgroup, we can further distinguish apical initiator caspases (caspase-8, -9 and -10) from downstream executioner caspases (caspase-3, -6 and -7) that are activated by the initiator caspases and execute apoptosis. However, as this review will show, "pro-apoptotic" caspases can also function in inflammatory responses, while activation of traditional "inflammatory" caspases can induce apoptosis [40, 41]. Furthermore, caspase-1 is involved in pyroptosis, an inflammatory form of cell death [42]. Caspase-14 mediates keratinocyte differentiation and may well be the only remaining truly non-apoptotic caspase [43]. Another classification divides caspases in a group with long (inflammatory and initiator caspases, caspase-2) or short prodomains (executioner caspases, caspase-14; Figure 2). The former group is usually activated by dimerization, which is obtained by the recruitment of caspases into oligomeric activation platforms. Adaptor molecules from these platforms can specifically bind to the death-effector domain (DED) or CARD domain in the prodomains of caspases, mediating a local increase in caspase concentration and proximity-induced activation [38]. Known activation platforms are the death-inducing signaling complex (DISC; in case of caspase-8 and -10), the apoptosome (in case of caspase-9), the PIDDosome (in case of caspase-2) and the inflammasomes (in case of caspase-1 and -5) [26, 38]. In contrast, activation of the executioner caspases, which occur as inactive dimers, requires cleavage by the initiator caspases in the unstructured linker region between the p20 and p10 subunits [38]. Caspase-14 needs both cleavage and dimerization for *in vitro* activation [44], but the upstream activator of caspase-14 is yet to be identified. Although caspase activation is often followed by its (auto)proteolytic maturation, it is important to note that maturation does not have to occur *per se* and that maturation alone is not able to generate an enzymatically active caspase [38]. For instance, caspase-8 can dimerize in the absence of maturation, which generates an active form of caspase-8 that is involved in T cell proliferation and activation. However, this form does not induce cell

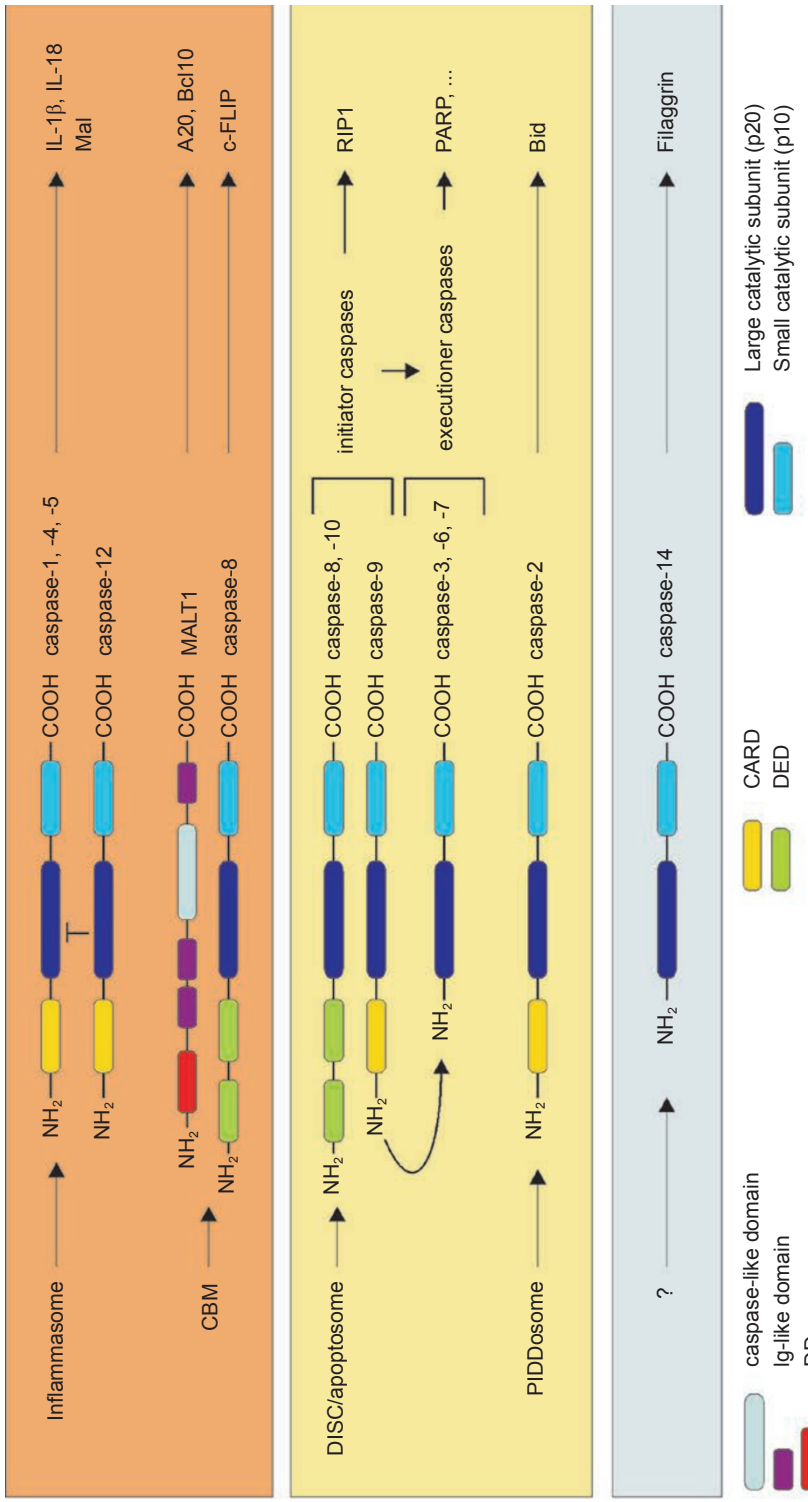

Figure 2 General function and structure of caspases and the paracaspase MALT1. Caspases can be classified on the basis of their function in apoptosis, inflammation or differentiation, which is associated with the cleavage of specific substrates. All caspases are characterized by a catalytic domain that can give rise to large and small subunits upon processing, and an N-terminal prodomain of variable length. The long prodomain caspases contain specific protein-protein interaction domains (CARD, DED) that are involved in the recruitment of caspases in specific multiprotein caspase-activation platforms (inflammasome, CBM (CARMA1/Bcl10/MALT1), apoptosome, PIDDosome). The paracaspase MALT1 shares with caspases a caspase-like domain, but cleaves its substrates after arginine instead of aspartic acid in the case of caspases.

death, which requires matured, cleaved caspase-8 [45].

The caspase-1, -4, -5 and -12 gene cluster: regulators of NF-κB and cytokine maturation

Caspase-1, -4, -5 and -12 are located in the same gene cluster and have been suggested to be functionally related. Caspase-1 has initially been identified as the protease responsible for proteolytic processing and activation of the proform of IL-1β [46]. This IL-1β processing function is believed to mediate in large part the pro-inflammatory function of caspase-1. However, overexpression studies indicated the potential of caspase-1 to also signal NF-κB activation (Table 1). This activity does not require its catalytic activity, but is rather mediated by its CARD domain and interaction with the kinase RIP2. Also, overexpression of the caspase-1-like protein COP, which in contrast to caspase-1 only consists of a CARD domain, is able to activate NF-κB via RIP2 [47, 48]. The role of caspase-1 in NF-κB signaling was further suggested by the finding that peritoneal macrophages from caspase-1-deficient mice, or cells treated with the caspase-1 inhibitor YVAD-cmk, show impaired NF-κB activation and TNF production in response to TLR2 or TLR4 stimulation [49, 50]. Catalytically inactive caspase-1 corrected the depressed NF-κB activity of the caspase-1-deficient cells [50], indicating that its NF-κB promoting function is independent of its catalytic activity. In contrast, caspase-1-mediated cleavage of the TLR adaptor protein Mal has been suggested to be critical for TLR2- and TLR4-induced NF-κB activation by exposing a critical interaction pocket of Mal [49, 51]. Recent studies, however, suggest that caspase-1-mediated Mal cleavage disrupts a critical protein-protein interaction site of its TIR domain, which mediates binding to TLR4 [52]. Interestingly, this finding is in line with the findings of Sarkar *et al*. [50], who showed that the wild-type caspase-1 effect on NF-κB is further augmented by inhibition of caspase-1 activity with YVAD-cmk. All together, these studies indicate a complex role of caspase-1 in the regulation of NF-κB.

Caspase-12, which is mainly located in the endoplasmic reticulum, has been reported to be involved in apoptotic and inflammatory pathways [53]. In most humans, it is present in a non-functional form due to a very recent loss-of-function mutation in the human lineage [54, 55]. Genetic and population analysis revealed that the functional form of caspase-12 is present in 20% to 30% of the African populations, while completely absent in Europe and Asia [56]. The two loss-of-function alleles present in humans encode either a short CARD-only or a catalytically inactive full-length form of caspase-12, which both seem to be inhibitory for caspase-1. Also, rodent caspase-12 has acquired a mutation in its catalytic pocket that renders it an extremely inefficient enzyme. Macrophages from caspase-12-deficient mice produce more IL-1β and IL-18, two pro-inflammatory cytokines processed and matured by caspase-1 [57]. Caspase-12 has been reported to inhibit the mucosal antimicrobial response independently from its effect on caspase-1. In this case, caspase-12 deficiency enhances the production of antimicrobial peptides and cytokines by intestinal epithelial cells in response to enteric pathogens via an effect on the Nod pathway. Mechanistically, caspase-12 was shown to block NF-κB activation by binding to RIP2 and displacing TRAF6 from the RIP2 signaling complex [58].

Caspase-4 and -5 are only present in humans and are supposed to be the functional homologs of caspase-11 in the mouse. Although they have been proposed to function in inflammatory pathways, their function is still largely unclear. LPS-induced NF-κB activation was found to be impaired in caspase-4-deficient THP-1 monocytic cell lines. LPS stimulation leads to the interaction of endogenous caspase-4 and TRAF6 via a TRAF6-binding motif in caspase-4 [59]. How this interaction affects the NF-κB activating potential of TRAF6 is still unknown.

Caspase-8 and -10: burn out or fade away?

Caspase-8 and caspase-10 have been mainly characterized as initiator caspases in death receptor signaling to apoptosis. Recruitment of caspase-8 and -10 to the DISC, which is assembled upon triggering of several death receptors, such as TNF receptor 1, Fas and TRAIL, induces pro-caspase homodimerization that is followed by autoproteolytic cleavage [38, 60-62]. The matured form subsequently cleaves and activates downstream apoptotic executioner caspases such as caspase-3. It is not fully clear whether caspase-8 or -10 activation occurs by inducible homodimerization or results from heterodimerization with other signaling components. In this context, heterodimerization with c-FLIP, which is a catalytically inactive homolog of caspase-8 and -10, is known to also activate caspase-8 and -10 [63]. On the other hand, c-FLIP has also been reported to inhibit cell death via competitive binding to adaptor molecules in the DISC [64]. Apart from their well-known function in apoptosis, caspase-8, -10 and c-FLIP have also been implicated in NF-κB activation. Initial studies demonstrated that overexpression of these proteins in HEK293T cells induces NF-κB activation, which depends on their DED in the N-terminal prodomain [65, 66]. Prodomain-only isoforms of caspase-8 and -10 that are generated by alternative splicing and have lost their apoptosis-inducing potential

Table 1 Overview of caspases and the experimental evidence for their involvement in the activation (+) or inhibition (-) of NF-κB

Caspase	NF-κB	Pathway	Evidence	Signaling partners	Proteolytic activity required	Cell type	References
caspase-1	+	TLR2, TLR4	KO, caspase inhibitor	Mal	Yes	macrophages	49-52
	+		overexpression	RIP2	No	HEK293T	47, 48
caspase-2	+		overexpression	RIP1, TRAF2	No	HEK293T	88
caspase-3	–		*in vitro* cleavage, overexpression	IκBα	Yes	chicken spleen cells	93
	–	TNF, naphtoquinone	caspase inhibitor, uncleavable mutant	p65	Yes	endothelial cells, HeLa	94, 95
	–	VP16,TNF	silencing, overexpression, mutants	Mst1, RCC1, p65	Yes	HeLa	96
caspase-4	+	LPS	KO	TRAF6	?	macrophages	59
caspase-8	+	TCR	allele variants, primary cells, mutant cell line	Bcl10, MALT1, TRAF6, c-FLIP	Yes	T cells	45, 78, 81, 82, 83
	+		overexpression	RIP1, NIK, TRAFs, IKK	No	HEK293T, MCF7, HeLa	65, 66, 68
	+	LPS	KO		?	B cells	79
	+	dsRNA	silencing, KO		?	MEF, HEK293T	74
	+	TRAIL	caspase inhibitor, silencing, overexpression	IκBα	Yes	HEK293T, HeLa	73
	+	MDP	silencing, caspase inhibitor		No	HEK293T-derived	75
caspase-10	+	TNF	overexpression	RIP1, NIK1, RIOK3	No	HEK293T, MCF7	65, 70
	+	dsRNA	silencing		?	MEF, HEK293T	74
caspase-12	–	MDP	overexpression, silencing	RIP2, TRAF6	No	HEK293T, HT29	58
MALT1	+		overexpression	Bcl10	Yes	HEK293T	101
	+		*in vitro* reconstitution	Bcl10, TRAF6, TAK1, NEMO	No	cell-free	97
	+	antigen receptors	KO, silencing, overexpression	A20	Yes	T cells, B cells	98, 99, 119, 120

Caspase	NF-κB	Pathway	Evidence	Signaling partners	Proteolytic activity required	Cell type	References
	+	LPS	silencing	IRAK1, Bcl10, TRAF6, TAK1	?	macrophages	108
	+	zymosan	KO	CARD9, Bcl10	?	myeloid cells	109
	+	ITAM receptors	KO	CARMA1, Bcl10	?	NK cells	110
	+	LPA	KO	Bcl10, PKC	?	MEF	112
	+	IgE	KO		?	*in vivo*, mast cells	111
	+	IL-8	silencing	CARD9, Bcl10	?	SVEC-10 murine endothelial cells	113
	+	angiotensin II	silencing, mutant expression	CARD9, Bcl10	?	HepG2	114
	+	BAFF	KO and crosses to BAFF-Tg, silencing	TRAF3	?	B cells, BJAB	115
	+	SDF-1α	silencing	CARD9, Bcl10	?	oral squamous cell carcinoma	116

still activate NF-κB, demonstrating that the apoptosis- and NF-κB-activating properties of these caspases can be separated by alternative splicing [67]. Caspase-8, -10 and c-FLIP have been shown to co-immunoprecipitate with RIP1, NIK, TRAFs and IKKs upon overexpression in HEK293T cells [65, 68], providing a possible mechanistic link. *In vitro* pull-down assays with recombinant proteins showed a direct interaction with RIP1, NIK and TRAF2, whereas the binding of IKKs is an indirect effect [69]. Although NIK is mainly known for its role in the non-canonical NF-κB signaling pathway leading to p100 processing, the latter was not induced by caspase-8 or -10 [69]. Overexpression of the atypical protein kinase RIOK3, which has recently been proposed as an endogenous inhibitor of TNF-induced NF-κB activation, was shown to prevent caspase-10-induced NF-κB activation by competing with RIP1 and NIK for binding to the caspase-10 prodomain [70]. Whereas the above-mentioned function of caspase-8 and -10 in NF-κB activation is independent of their catalytic activity, the latter may still affect the NF-κB response in other ways. For example, proteolytic cleavage of RIP1 by caspase-8 during TNF-induced apoptosis abrogates the NF-κB stimulating function of RIP1 and promotes apoptosis [71]. Similarly, caspase-8 cleaves RIP1 in monocytes undergoing macrophage differentiation, preventing sustained NF-κB activation [72]. On the other hand, caspase-8-mediated cleavage of IκBα, followed by its proteasome-dependent degradation, has been suggested to be involved in the late phase of TRAIL-induced NF-κB activation [73]. Since many of the above mentioned data are based on overexpression, the physiological role of caspase-8 and -10 in death receptor-induced NF-κB activation remains to be proven.

Apart from NF-κB signaling initiated by death receptors, caspase-8 and -10 have also been implicated in NF-κB signaling mediated by the intracellular retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (Mda5) receptors, which recognize double-stranded RNA and play an essential role in antiviral immune responses. RIG-I and Mda5 function via the adaptor protein mitochondrial antiviral signaling (MAVS), which can bind FADD, which in turn binds caspase-8 and -10. Double-stranded RNA stimulation of HEK293T cells induced NF-κB activation that was associated with the processing of caspase-8 and -10. Analogously, overexpression of the caspase-8 or -10 DED, but not the full-length form, also activated NF-κB, indicating a signaling role for the processed forms of caspase-8 and -10. Furthermore, NF-κB activation in response to double-stranded RNA stimulation or transfection of a constitutively active RIG-I mutant or Mda5 is significantly impaired in HEK293T cells in which caspase-8 or -10 is knocked down, or in murine embryonic fibroblasts derived from caspase-8-deficient mice [74]. In contrast, IFN-β promoter activation was normal in caspase-8-deficient cells. These results indicate that caspase-8 and -10 are essential components that mediate NF-κB-dependent inflammatory responses in antiviral signaling initiated by the intracellular receptors RIG-I and Mda5. Similarly, caspase-8 has also been suggested to mediate NF-κB activation by intracellular NLRs such as IPAF [75], which sense specific bacterial products and signal via ASC. Considering the structural similarity of FADD and ASC, it is likely that ASC plays an equivalent role to FADD as an adaptor protein that acts as a bridge between an NLR and caspase-8. However, further work on the role of ASC and caspase-8 in NLR-induced NF-κB signaling is needed since studies involving ASC-deficient mice have indicated that ASC is not essential for the expression of cytokine genes and NF-κB activation induced by microbial infection in murine macrophages [76].

Caspase-8 is essential for T cell activation and IL-2-dependent proliferation via mechanisms that are still poorly defined [77]. In this context, a role for caspase-8 in TCR-induced NF-κB activation has been claimed, but a lot of controversy remains. Lenardo and colleagues [78] reported that T cells from patients with an inactivating mutation in caspase-8, as well as caspase-8-deficient Jurkat T cells, are defective in IκBα degradation and NF-κB nuclear translocation after TCR stimulation. Additionally, B cells from caspase-8 mutant patients are impaired in NF-κB activation upon BCR or LPS triggering, and NK cells from these patients are defective in FcγRIII- but not CD40-induced NF-κB translocation. Using mice with B cell-specific inactivation of caspase-8, the role of caspase-8 in LPS-induced NF-κB signaling in B cells could be confirmed. Furthermore, in response to TLR4 triggering, caspase-8 is transiently recruited to a complex containing IKKα/β [79]. However, T cells from mice with a caspase-8 deletion that is restricted to the T cell lineage did show very controversial effects. Defective NF-κB activation in response to TCR stimulation was observed in a study by Su *et al*. [78], but no effect could be observed in two other studies [77, 80], despite the fact that all three studies confirmed the role of caspase-8 in T cell proliferation. The explanation for these differences remains to be found. Also, the molecular mechanism by which caspase-8 could activate NF-κB is still unclear. Reconstitution experiments in the caspase-8-deficient Jurkat T cell line revealed that enzymatically active full-length caspase-8 is required for NF-κB signaling [45, 78]. Co-immunoprecipitation experiments show that TCR stimulation promotes the formation of a complex of caspase-8 with Bcl10 and MALT1 (see also below). Recruitment

of TRAF6 to this complex promotes its movement to lipid rafts. This is followed by IKK recruitment and NF-κB activation [78, 81, 82]. Why enzymatic activity of caspase-8 is required for TCR-induced NF-κB activation is still unclear. It is conceivable that caspase-8 cleaves an unknown substrate, promoting NF-κB activation. One candidate is the caspase-8 regulator c-FLIP as it was shown that c-FLIP can be cleaved by active, full-length caspase-8 with the formation of p43 and p22 fragments, which can trigger NF-κB activity by more avidly binding to RIP1 and TRAF2, or IKKγ, respectively [82, 83]. However, the involvement of c-FLIP in TCR-induced NF-κB signaling is still controversial, as no obvious defects in TCR-induced NF-κB activation were reported in thymocytes from mice with a conditional c-FLIP deletion in the T cell lineage [84, 85]. Alternatively, caspase-8 may have a different conformation in its active state that enhances the binding and/or ubiquitin ligase activity of TRAF6, without cleavage of a specific substrate. Another intriguing question is how caspase-8 becomes activated upon TCR stimulation without triggering cell death. Kawadler *et al*. [86] demonstrated that MALT1 controls caspase-8 activation upon TCR triggering through direct association. This promotes limited proteolytic processing and generates an enzymatically active caspase-8, which has full activity towards c-FLIP, but a diminished activity towards caspase-3 [86]. Furthermore, fully processed caspase-8 induced upon triggering of death receptors is found almost exclusively in the cytosolic compartment, where it is highly active towards executioner caspases. In contrast, enzymatically active, full-length caspase-8, which is formed upon TCR engagement, is located at the lipid rafts, demonstrating a clear spatial difference between these forms of activated caspase-8 [87].

An NF-κB inhibitory function of "apoptotic" caspases-2, -3 and -7

Caspase-2 is the most conserved caspase, and displays features common to both the initiator and executioner caspases. A non-catalytic mechanism of caspase-2-mediated NF-κB activation, involving RIP1 and TRAF2, was originally proposed based on overexpression studies [88]. However, a direct role for endogenously expressed caspase-2 in NF-κB signaling has not yet been demonstrated. One of the best known activities of caspase-2 is its role in p53-dependent cell death via the so-called PIDDosome, which can either signal NF-κB activation or alternatively trigger caspase-2-mediated cell death [89, 90] (Figure 1). NF-κB activation by PIDD involves ATM-dependent NEMO phosphorylation, but is independent of caspase-2 [91]. Recently, caspase-2 was shown to repress survivin gene expression in tumor cells by cleaving RIP1 and inhibiting NF-κB activity [92], indicating that caspase-2 may also promote cell death by inhibiting NF-κB-dependent cell survival. This is similar to the cleavage of RIP1 by caspase-8 in response to TNF receptor stimulation [71] or in the case of macrophage differentiation [72]. Also, caspase-3 has been shown to interfere with NF-κB activation. In this case, caspase-3 cleaves IκBα, generating a cleavage fragment that potentially acts as a constitutive inhibitor of NF-κB. The caspase-3-mediated cleavage of IκBα is inhibited by IκBα phosphorylation, indicating antagonistic effects between pro-apoptotic and survival signaling pathways [93]. Similarly, the NF-κB subunit p65 is also cleaved by caspase-3 in TNF-stimulated cells [94, 95]. Apoptotic caspases can also inhibit the nuclear import of p65, among others, by immobilization of regulator or chromatin condensation 1 (RCC1) via cleavage of Mst1, leading to lowered nuclear Ran levels [96]. Finally, the caspase-3-related protease caspase-7 has several protein substrates in common with caspase-3, but a role for caspase-7 in NF-κB regulation has not yet been reported.

The paracaspase MALT1: working double shifts as a scaffold and a guard in multiple pathways

MALT1 is a cytosolic signaling molecule that plays a key role in NF-κB signaling in response to antigen receptor stimulation (reviewed in [36, 97]). MALT1-deficient mice are defective in antigen receptor-induced lymphocyte activation [98, 99]. MALT1 consists of an N-terminal DD followed by two immunoglobulin (Ig)-like domains, a caspase-like domain and a C-terminal Ig-like domain (Figure 2), and was originally identified as the target of recurrent translocations in a large fraction of MALT lymphomas [100]. These translocations result in a chimeric protein (API2-MALT1) in which the N-terminus is contributed by the apoptosis inhibitor cIAP2, and the C-terminus (lacking the DD) by MALT1. Initial studies using overexpression of API2-MALT1 or MALT1 illustrated the NF-κB activating potential of MALT1 [101]. Whereas API2-MALT1 overexpression strongly activates NF-κB, MALT1-induced NF-κB activation requires co-expression of the MALT1-binding protein Bcl10. Most likely, this reflects the requirement for oligomerization, which can be imposed by either the fused BIR domains of cIAP2 or the binding to Bcl10. However, it cannot be excluded that binding of other proteins to the cIAP2 part of API2-MALT1 also contributes to NF-κB signaling. The role of MALT1 in NF-κB signaling has for a long time been solely attributed to its scaffolding function. Several binding partners for MALT1 have been

identified. Bcl10 binds constitutively to MALT1, and it is thought that the Bcl10-MALT1 complex is recruited to the membrane-associated adaptor protein CARMA1 upon antigen receptor stimulation. This requires the PKC-mediated phosphorylation of CARMA1, which results in a conformational change that allows Bcl10-MALT1 binding and the formation of the so-called CBM complex that is found in lipid rafts [102]. The mechanism by which this complex links to IKK activation is still not completely understood. One way is through the recruitment of the ubiquitin ligase TRAF6, which activates TAK1 and IKK [97, 103]. TRAF6 can ubiquitinate MALT1 and it has been suggested that this provides a docking site for IKKγ and thereby mediates the recruitment of the IKK complex [104]. MALT1 ubiquitination has also been attributed to an intrinsic ubiquitin ligase activity of MALT1 [105], but this is much debated. Finally, also ubiquitination of Bcl10 has been proposed to contribute to the recruitment of the IKK complex [106]. Interestingly, Bcl10 and MALT1 were found to differentially regulate BCR-induced activation of p65 and c-Rel. In this study, Bcl10 was essential for the recruitment of IKK into lipid rafts and for the activation of RelA and c-Rel. In contrast, MALT1 was not involved in IKK recruitment or activation and was dispensable for p65 induction, but selectively activates c-Rel through an as-of-yet unknown mechanism [107]. Apart from TCR and BCR pathways, MALT1 has been implicated in NF-κB signaling initiated by several other receptors, such as TLR4 [108], Dectin-1 [109], NK cell receptors [110], Fc epsilon RI [111], lysophosphatidic acid receptor [112], IL-8 receptor [113], angiotensin II receptor [114], BAFF receptor [115] and CXCR4 [116], featuring at least two distinct CBM complexes containing CARMA1 (also known as CARD11) or CARD9 [117]. The annotation of MALT1 as a paracaspase was based on its sequence similarity to caspases and an additional family of caspase-like proteins known as metacaspases, which have variable biological functions in yeast, plants and parasites [118]. The presence of two conserved residues predicted to be important for catalytic activity in various caspases suggested that MALT1 would also have caspase-like proteolytic activity. Moreover, MALT1 constructs mutated in the predicted active site Cys showed a significant reduction in their capacity to activate NF-κB in transfected HEK293T cells [101]. The proteolytic activity of MALT1, however, remained elusive and several attempts to demonstrate proteolytic activity remained unsuccessful until recently when it was found that Bcl10 [119] and the ubiquitin-editing protein A20 [120], also known as TNFAIP3, were cleaved by MALT1 in response to TCR and BCR stimulation. MALT1 cleaves its substrates after arginine, which is different from caspases, which are aspartic acid specific. Optimal conditions for inducing MALT1 oligomerization seemed to be crucial for the formal demonstration of proteolytic activity of MALT1 *in vitro*. In contrast to both the "inflammatory" and the "apoptotic" caspases, overexpression of artificially dimerized MALT1 with high proteolytic activity does not lead to any notable cell death (our unpublished data). Catalytically inactive MALT1 can still activate NF-κB, but with a much lower efficiency [120]. Also, treatment of cells with a cell-permeable MALT1 inhibitor peptide showed that the proteolytic activity of MALT1 is required for optimal NF-κB activation in T cells [119]. This is consistent with MALT1-mediated proteolytic inactivation of A20, which is known to negatively regulate NF-κB activation in response to distinct receptors by de-ubiquitinating specific signaling molecules such as RIP1, TRAF6, IKKγ and MALT1 [121, 122]. MALT1-mediated cleavage of A20 separates its N-terminal de-ubiquitinating domain from the C-terminal substrate-binding domain, which led to the suggestion that A20 cleavage might serve to remove the de-ubiquitinating domain from A20 molecules associated with specific NF-κB signaling molecules, thereby preserving activating ubiquitination events. Recently, A20 cleavage by MALT1 was found to induce the cytosolic release of the N-terminal fragment of A20 from a particulate and insoluble cell fraction, suggesting that loss of compartmentalization might also contribute to MALT1-mediated dampening of A20 function [123]. Bcl10 cleavage could not be linked with altered NF-κB signaling, but is required for TCR-induced cell adhesion to fibronectin [119]. Collectively, the present data suggest that MALT1 controls T cell activation and the strength of the NF-κB response through both its adaptor and protease functions. As mentioned above, also caspase-8 binding to MALT1, inducing caspase-8 cleavage of c-FLIP, can initiate a pathway that contributes to NF-κB activation. Although many questions remain, therapeutic targeting of the protease activity of MALT1 might become a useful approach for the treatment of autoimmune or inflammatory diseases, for the prevention of transplant rejection and the treatment of B cell lymphomas such as MALT lymphoma and ABC-type DLBCL associated with MALT1 dysfunction. *In vitro* studies with a peptide-based, cell-permeable MALT1 inhibitor have already proven the potential of MALT1 as a therapeutic target against a subclass of B cell lymphomas that depend on constitutive NF-κB activity [124, 125].

Concluding remarks

In comparison to phosphorylation and ubiquitination,

the role of proteolytic cleavage in inflammatory signal transduction is still in its infancy. For several caspases, their function in NF-κB signaling appears to be primarily as scaffold proteins recruiting other signaling mediators and their proteolytic activity often appears to represent an antagonistic outcome to the one leading to NF-κB activation. Classical "apoptotic" proteases such as caspase-3 also seem to have the ability to directly block various stages of NF-κB signaling, which presumably enhances their apoptotic potential. The functional role of caspases is therefore most likely the result of a complex relationship between cell death, cytokine processing, NF-κB signaling and other events. It should also be mentioned that for many caspases evidence for a role in NF-κB signaling is still based on overexpression studies. Taking into account that caspase signaling occurs in highly specific subcomplexes targeting specific sites at specific proteins, the physiological relevance of findings based on overexpression should be taken with caution. Despite that only a minor fraction of the total cell content of a specific protein is modified by protease cleavage, the specific context in which it is cleaved can have profound effects on the signaling outcome, as demonstrated by the opposing roles of caspase-8. A major future challenge will be to define these complexes and contexts, how they are regulated, what they regulate and how we can specifically utilize and manipulate this for the next-generation anti-inflammatory treatments. Until now, much of our knowledge on the role of caspases in NF-κB signaling is sometimes very controversial. A striking exception is the paracaspase MALT1, whose NF-κB activating function is key in T and B cell activation and proliferation, and whose proteolytic activity is critical for a full NF-κB response. In particular, the possibility to modulate inflammation or lymphomagenesis through specific inhibition of MALT1 may hold the promise of highly specific therapeutic strategies. Knockout studies in mice have implicated an important role of several caspases and MALT1 in normal homeostasis, development and disease, but it is still largely unclear to what extent NF-κB modulation contributes to the observed phenotype. The generation and analysis of knockin mice carrying catalytically inactive caspase mutants might give us a clue.

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References

1 Deribe YL, Pawson T, Dikic I. Post-translational modifications in signal integration. *Nat Struct Mol Biol* 2010; **17**:666-672.
2 Keller M, Rüegg A, Werner S, Beer H. Active caspase-1 is a regulator of unconventional protein secretion. *Cell* 2008; **132**:818-831.
3 Kurokawa M, Kornbluth S. Caspases and kinases in a death grip. *Cell* 2009; **138**:838-854.
4 Howley B, Fearnhead HO. Caspases as therapeutic targets. *J Cell Mol Med* 2008; **12**:1502-1516.
5 Vucic D, Dixit VM. Masking MALT1: the paracaspase's potential for cancer therapy. *J Exp Med* 2009; **206**:2309-2312.
6 Yazdi AS, Guarda G, D'Ombrain MC, Drexler SK. Inflammatory caspases in innate immunity and inflammation. *J Innate Immun* 2010; **2**:228-237.
7 Maelfait J, Beyaert R. Non-apoptotic functions of caspase-8. *Biochem Pharmacol* 2008; **76**:1365-1373.
8 Lamkanfi M, Festjens N, Declercq W, Vanden Berghe T, Vandenabeele P. Caspases in cell survival, proliferation and differentiation. *Cell Death Differ* 2007; **14**:44-55.
9 Vallabhapurapu S, Karin M. Regulation and function of NF-κB transcription factors in the immune system. *Annu Rev Immunol* 2009; **27**:693-733.
10 Oeckinghaus A, Ghosh S. The NF-κB family of transcription factors and its regulation. *Cold Spring Harb Perspect Biol* 2009; **1**:a000034.
11 Baldwin AS Jr. The NF-κB and IκB proteins: new discoveries and insights. *Annu Rev Immunol* 1996; **14**:649-683.
12 Ghosh S, May MJ, Kopp EB. NF-κB and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* 1998; **16**:225-260.
13 Chen ZJ, Parent L, Maniatis T. Site-specific phosphorylation of IκBα by a novel ubiquitination-dependent protein kinase activity. *Cell* 1996; **84**:853-862.
14 Mercurio F, Zhu H, Murray BW, *et al*. IKK-1 and IKK-2: cytokine-activated IκB kinases essential for NF-κB activation. *Science* 1997; **278**:860-866.
15 Woronicz JD, Gao X, Cao Z, Rothe M, Goeddel DV. IκB kinase-β: NF-κB activation and complex formation with IκB kinase-α and NIK. *Science* 1997; **278**:866-869.
16 Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M. The IκB kinase complex (IKK) contains two kinase subunits, IKKα and IKKβ, necessary for IκB phosphorylation and NF-κB activation. *Cell* 1997; **91**:243-252.
17 Li Q, Van Antwerp D, Mercurio F, Lee KF, Verma IM. Severe liver degeneration in mice lacking the IκB kinase 2 gene. *Science* 1999; **284**:321-325.
18 Li ZW, Chu W, Hu Y, *et al*. The IKKβ subunit of IκB kinase (IKK) is essential for nuclear factor κB activation and prevention of apoptosis. *J Exp Med* 1999; **189**:1839-1845.
19 Yang J, Lin Y, Guo Z, *et al*. The essential role of MEKK3 in TNF-induced NF-κB activation. *Nat Immunol* 2001; **2**:620-624.

20 Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* 2001; **412**:346-351.
21 Shim J, Xiao C, Paschal AE, *et al*. TAK1, but not TAB1 or TAB2, plays an essential role in multiple signaling pathways *in vivo*. *Genes Dev* 2005; **19**:2668-2681.
22 Yamazaki K, Gohda J, Kanayama A, *et al*. Two mechanistically and temporally distinct NF-κB activation pathways in IL-1 signaling. *Sci Signal* 2009; **2**:ra66.
23 Senftleben U, Cao Y, Xiao G, *et al*. Activation by IKKα of a second, evolutionary conserved, NF-κB signaling pathway. *Science* 2001; **293**:1495-1499.
24 Xiao G, Fong A, Sun S. Induction of p100 processing by NF-κB-inducing kinase involves docking IκB kinase α (IKKα) to p100 and IKKα-mediated phosphorylation. *J Biol Chem* 2004; **279**:30099-30105.
25 Janssens S, Tschopp J. Signals from within: the DNA-damage-induced NF-κB response. *Cell Death Differ* 2006; **13**:773-784.
26 Tinel A, Tschopp J. The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. *Science* 2004; **304**:843-846.
27 Legarda-Addison D, Hase H, O'Donnell MA, Ting AT. NEMO/IKKγ regulates an early NF-κB-independent cell-death checkpoint during TNF signaling. *Cell Death Differ* 2009; **16**:1279-1288.
28 Tokunaga F, Sakata S, Saeki Y, *et al*. Involvement of linear polyubiquitylation of NEMO in NF-κB activation. *Nat Cell Biol* 2009; **11**:123-132.
29 Kawagoe T, Sato S, Matsushita K, *et al*. Sequential control of Toll-like receptor-dependent responses by IRAK1 and IRAK2. *Nat Immunol* 2008; **9**:684-691.
30 Hacker H, Redecke V, Blagoev B, *et al*. Specificity in Toll-like receptor signaling through distinct effector functions of TRAF3 and TRAF6. *Nature* 2006; **439**:204-207.
31 Cusson-Hermance N, Khurana S, Lee TH, Fitzgerald KA, Kelliher MA. Rip1 mediates the Trif-dependent toll-like receptor 3- and 4-induced NF-κB activation but does not contribute to interferon regulatory factor 3 activation. *J Biol Chem* 2005; **280**:36560-36566.
32 Oganesyan G, Saha SK, Guo B, *et al*. Critical role of TRAF3 in the Toll-like receptor-dependent and -independent antiviral response. *Nature* 2006; **439**:208-211.
33 Inohara N, Koseki T, del Peso L, *et al*. Nod1, an Apaf-1-like activator of caspase-9 and nuclear factor-κB. *J Biol Chem* 1999; **274**:14560-14567.
34 Ogura Y, Inohara N, Benito A, Chen FF, Yamaoka S, Nunez G. Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-κB. *J Biol Chem* 2001; **276**:4812-4818.
35 McCarthy JV, Ni J, Dixit VM. RIP2 is a novel NF-κB-activating and cell death-inducing kinase. *J Biol Chem* 1998; **273**:16968-16975.
36 Thome M. CARMA1, BCL-10 and MALT1 in lymphocyte development and activation. *Nat Rev Immunol* 2004; **4**:348-359.
37 Xia ZP, Sun L, Chen X, *et al*. Direct activation of protein kinases by unanchored polyubiquitin chains. *Nature* 2009; **461**:114-119.
38 Pop C, Salvesen GS. Human caspases: activation, specificity, and regulation. *J Biol Chem* 2009; **284**:21777-21781.
39 Lippens S, VandenBroecke C, Van Damme E, Tschachler E, Vandenabeele P, Declercq W. Caspase-14 is expressed in the epidermis, the choroid plexus, the retinal pigment epithelium and thymic Hassall's bodies. *Cell Death Differ* 2003; **10**:257-259.
40 Kamada S, Funahashi Y, Tsujimoto Y. Caspase-4 and caspase-5, members of the ICE/CED-3 family of cysteine proteases, are CrmA-inhibitable proteases. *Cell Death Differ* 1997; **4**:473-478.
41 Miura M, Zhu H, Rotello R, Hartwieg EA, Yuan J. Induction of apoptosis in fibroblasts by IL-1β-converting enzyme, a mammalian homolog of the *C. elegans* cell death gene ced-3. *Cell* 1993; **75**:653-660.
42 Hersh D, Monack DM, Smith MR, Ghori N, Falkow S, Zychlinsky A. The Salmonella invasin SipB induces macrophage apoptosis by binding to caspase-1. *Proc Natl Acad Sci USA* 1999; **96**:2396-2401.
43 Denecker G, Hoste E, Gilbert B, *et al*. Caspase-14 protects against epidermal UVB photodamage and water loss. *Nat Cell Biol* 2007; **9**:666-674.
44 Mikolajczyk J, Scott FL, Krajewski S, Sutherlin DP, Salvesen GS. Activation and substrate specificity of caspase-14. *Biochemistry* 2004; **43**:10560-10569.
45 Leverrier S, Salvesen GS, Walsh CM. Enzymatically active single chain caspase-8 maintains T-cell survival during clonal expansion. *Cell Death Differ* 2010; doi: 10.1038/cdd.2010.69.
46 Howard AD, Kostura MJ, Thornberry N, *et al*. IL-1-converting enzyme requires aspartic acid residues for processing of the IL-1β precursor at two distinct sites and does not cleave 31-kDa IL-1α. *J Immunol* 1991; **147**:2964-2969.
47 Lamkanfi M, Kalai M, Saelens X, Declercq W, Vandenabeele P. Caspase-1 activates nuclear factor of the κ-enhancer in B cells independently of its enzymatic activity. *J Biol Chem* 2004; **279**:24785-24793.
48 Lamkanfi M, Denecker G, Kalai M, *et al*. INCA, a Novel Human Caspase recruitment domain protein that inhibits interleukin-1β generation. *J Biol Chem* 2004; **279**:51729-51738.
49 Miggin SM, Pålsson-McDermott E, Dunne A, *et al*. NF-κB activation by the Toll-IL-1 receptor domain protein MyD88 adapter-like is regulated by caspase-1. *Proc Natl Acad Sci USA* 2007; **104**:3372-3377.
50 Sarkar A, Duncan M, Hart J, Hertlein E, Guttridge DC, Wewers MD. ASC directs NF-κB activation by regulating receptor interacting protein-2 (RIP2) caspase-1 interactions. *J Immunol* 2006; **176**:4979-4986.
51 Núñez Miguel R, Wong J, Westoll JF, *et al*. A dimer of the Toll-like receptor 4 cytoplasmic domain provides a specific scaffold for the recruitment of signaling adaptor proteins. *PLoS ONE* 2007; **2**:e788.
52 Ulrichts P, Bovijn C, Lievens S, Beyaert R, Tavernier J, Peelman F. Caspase-1 targets the TLR adaptor Mal at a crucial TIR-domain interaction site. *J Cell Sci* 2010; **123**:256-265.
53 Nakagawa T, Zhu H, Morishima N, *et al*. Caspase-12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by amyloid-β. *Nature* 2000; **403**:98-103.

54 Fischer H, Koenig U, Eckhart L, Tschachler E. Human caspase 12 has acquired deleterious mutations. *Biochem Biophys Res Commun* 2002; **293**:722-726.
55 Kersse K, Vanden Berghe T, Lamkanfi M, Vandenabeele P. A phylogenetic and functional overview of inflammatory caspases and caspase-1-related CARD-only proteins. *Biochem Soc Trans* 2007; **35**:1508-1511.
56 Kachapati K, O'Brien TR, Bergeron J, Zhang M, Dean M. Population distribution of the functional caspase-12 allele. *Hum Mutat* 2006; **27**:975.
57 Saleh M, Mathison JC, Wolinski MK, *et al*. Enhanced bacterial clearance and sepsis resistance in caspase-12-deficient mice. *Nature* 2006; **440**:1064-1068.
58 LeBlanc PM, Yeretssian G, Rutherford N, *et al*. Caspase-12 modulates NOD signaling and regulates antimicrobial peptide production and mucosal immunity. *Cell Host Microbe* 2008; **3**:146-157.
59 Lakshmanan U, Porter AG. Caspase-4 interacts with TNF receptor-associated factor 6 and mediates lipopolysaccharide-induced NF-κB-dependent production of IL-8 and CC chemokine ligand 4 (macrophage-inflammatory protein-1). *J Immunol* 2007; **179**:8480-8490.
60 Boatright KM, Renatus M, Scott FL, *et al*. A unified model for apical caspase activation. *Mol Cell* 2003; **11**:529-541.
61 Peter ME, Krammer PH. Mechanisms of CD95 (APO-1/Fas)-mediated apoptosis. *Curr Opin Immunol* 1998; **10**:545-551.
62 Wang J, Chun HJ, Wong W, Spencer DM, Lenardo MJ. Caspase-10 is an initiator caspase in death receptor signaling. *Proc Natl Acad Sci USA* 2001; **98**:13884-13888.
63 Boatright KM, Deis C, Denault J, Sutherlin DP, Salvesen GS. Activation of caspases-8 and -10 by FLIP(L). *Biochem J* 2004; **382**:651-657.
64 Irmler M, Thome M, Hahne M, *et al*. Inhibition of death receptor signals by cellular FLIP. *Nature* 1997; **388**:190-195.
65 Chaudhary PM, Eby MT, Jasmin A, Kumar A, Liu L, Hood L. Activation of the NF-κB pathway by caspase 8 and its homologs. *Oncogene* 2000; **19**:4451-4460.
66 Hu WH, Johnson H, Shu HB. Activation of NF-κB by FADD, Casper, and caspase-8. *J Biol Chem* 2000; **275**:10838-10844.
67 Wang H, Wang P, Sun X, *et al*. Cloning and characterization of a novel caspase-10 isoform that activates NF-κB activity. *Biochim Biophys Acta* 2007; **1770**:1528-1537.
68 Chaudhary PM, Eby MT, Jasmin A, Hood L. Activation of the c-Jun N-terminal kinase/stress-activated protein kinase pathway by overexpression of caspase-8 and its homologs. *J Biol Chem* 1999; **274**:19211-19219.
69 Shikama Y, Yamada M, Miyashita T. Caspase-8 and caspase-10 activate NF-κB through RIP, NIK and IKKα kinases. *Eur J Immunol* 2003; **33**:1998-2006.
70 Shan J, Wang P, Zhou J, Wu D, Shi H, Huo K. RIOK3 interacts with caspase-10 and negatively regulates the NF-κB signaling pathway. *Mol Cell Biochem* 2009; **332**:113-120.
71 Lin Y, Devin A, Rodriguez Y, Liu ZG. Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. *Genes Dev* 1999; **13**:2514-2526.
72 Rébé C, Cathelin S, Launay S, *et al*. Caspase-8 prevents sustained activation of NF-κB in monocytes undergoing macrophagic differentiation. *Blood* 2007; **109**:1442-1450.
73 Rathore N, Matta H, Chaudhary PM. An evolutionary conserved pathway of nuclear factor-κB activation involving caspase-mediated cleavage and N-end rule pathway-mediated degradation of IκBα. *J Biol Chem* 2004; **279**:39358-39365.
74 Takahashi K, Kawai T, Kumar H, Sato S, Yonehara S, Akira S. Roles of caspase-8 and caspase-10 in innate immune responses to double-stranded RNA. *J Immunol* 2006; **176**:4520-4524.
75 Hasegawa M, Imamura R, Motani K, *et al*. Mechanism and repertoire of ASC-mediated gene expression. *J Immunol* 2009; **182**:7655-7662.
76 Mariathasan S, Newton K, Monack DM, *et al*. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* 2004; **430**:213-218.
77 Salmena L, Lemmers B, Hakem A, *et al*. Essential role for caspase 8 in T-cell homeostasis and T-cell-mediated immunity. *Genes Dev* 2003; **17**:883-895.
78 Su H, Bidère N, Zheng L, *et al*. Requirement for caspase-8 in NF-κB activation by antigen receptor. *Science* 2005; **307**:1465-1468.
79 Lemmers B, Salmena L, Bidère N, *et al*. Essential role for caspase-8 in Toll-like receptors and NFκB signaling. *J Biol Chem* 2007; **282**:7416-7423.
80 Ch'en IL, Beisner DR, Degterev A, *et al*. Antigen-mediated T cell expansion regulated by parallel pathways of death. *Proc Natl Acad Sci USA* 2008; **105**:17463-17468.
81 Bidère N, Snow AL, Sakai K, Zheng L, Lenardo MJ. Caspase-8 regulation by direct interaction with TRAF6 in T cell receptor-induced NF-κB activation. *Curr Biol* 2006; **16**:1666-1671.
82 Misra RS, Russell JQ, Koenig A, *et al*. Caspase-8 and c-FLIPL associate in lipid rafts with NF-κB adaptors during T cell activation. *J Biol Chem* 2007; **282**:19365-19374.
83 Golks A, Brenner D, Krammer PH, Lavrik IN. The c-FLIP-NH2 terminus (p22-FLIP) induces NF-κB activation. *J Exp Med* 2006; **203**:1295-1305.
84 Chau H, Wong V, Chen N, *et al*. Cellular FLICE-inhibitory protein is required for T cell survival and cycling. *J Exp Med* 2005; **202**:405-413.
85 Zhang N, He Y. An essential role for c-FLIP in the efficient development of mature T lymphocytes. *J Exp Med* 2005; **202**:395-404.
86 Kawadler H, Gantz MA, Riley JL, Yang X. The paracaspase MALT1 controls caspase-8 activation during lymphocyte proliferation. *Mol Cell* 2008; **31**:415-421.
87 Koenig A, Russell JQ, Rodgers WA, Budd RC. Spatial differences in active caspase-8 defines its role in T-cell activation versus cell death. *Cell Death Differ* 2008; **15**:1701-1711.
88 Lamkanfi M, D'hondt K, Vande Walle L, *et al*. A novel caspase-2 complex containing TRAF2 and RIP1. *J Biol Chem* 2005; **280**:6923-6932.
89 Kumar S. Caspase 2 in apoptosis, the DNA damage response and tumour suppression: enigma no more? *Nat Rev Cancer* 2009; **9**:897-903.
90 Vakifahmetoglu-Norberg H, Zhivotovsky B. The unpredictable caspase-2: what can it do? *Trends Cell Biol* 2010; **20**:150-159.
91 Janssens S, Tinel A, Lippens S, Tschopp J. PIDD mediates NF-κB activation in response to DNA damage. *Cell* 2005; **123**:1079-1092.
92 Guha M, Xia F, Raskett CM, Altieri DC. Caspase 2-mediated

tumor suppression involves survivin gene silencing. *Oncogene* 2010; **29**:1280-1292.
93 Barkett M, Xue D, Horvitz HR, Gilmore TD. Phosphorylation of IκB-α inhibits its cleavage by caspase CPP32 *in vitro*. *J Biol Chem* 1997; **272**:29419-29422.
94 Kang KH, Lee KH, Kim MY, Choi KH. Caspase-3-mediated cleavage of the NF-κB subunit p65 at the NH2 terminus potentiates naphthoquinone analog-induced apoptosis. *J Biol Chem* 2001; **276**:24638-24644.
95 Neuzil J, Schröder A, von Hundelshausen P, *et al*. Inhibition of inflammatory endothelial responses by a pathway involving caspase activation and p65 cleavage. *Biochemistry* 2001; **40**:4686-4692.
96 Wong C, Chan H, Ho C, *et al*. Apoptotic histone modification inhibits nuclear transport by regulating RCC1. *Nat Cell Biol* 2009; **11**:36-45.
97 Sun L, Deng L, Ea C, Xia Z, Chen ZJ. The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. *Mol Cell* 2004; **14**:289-301.
98 Ruefli-Brasse AA, French DM, Dixit VM. Regulation of NF-κB-dependent lymphocyte activation and development by paracaspase. *Science* 2003; **302**:1581-1584.
99 Ruland J, Duncan GS, Wakeham A, Mak TW. Differential requirement for Malt1 in T and B cell antigen receptor signaling. *Immunity* 2003; **19**:749-758.
100 Dierlamm J, Baens M, Wlodarska I, *et al*. The apoptosis inhibitor gene API2 and a novel 18q gene, MLT, are recurrently rearranged in the t(11;18)(q21;q21) associated with mucosa-associated lymphoid tissue lymphomas. *Blood* 1999; **93**:3601-3609.
101 Lucas PC, Yonezumi M, Inohara N, *et al*. Bcl10 and MALT1, independent targets of chromosomal translocation in malt lymphoma, cooperate in a novel NF-κB signaling pathway. *J Biol Chem* 2001; **276**:19012-19019.
102 Che T, You Y, Wang D, Tanner MJ, Dixit VM, Lin X. MALT1/paracaspase is a signaling component downstream of CARMA1 and mediates T cell receptor-induced NF-κB activation. *J Biol Chem* 2004; **279**:15870-15876.
103 Noels H, van Loo G, Hagens S, *et al*. A novel TRAF6 binding site in MALT1 defines distinct mechanisms of NF-κB activation by API2:MALT1 fusions. *J Biol Chem* 2007; **282**:10180-10189.
104 Oeckinghaus A, Wegener E, Welteke V, *et al*. Malt1 ubiquitination triggers NF-κB signaling upon T-cell activation. *EMBO J* 2007; **26**:4634-4645.
105 Zhou H, Du M, Dixit VM. Constitutive NF-κB activation by the t(11;18)(q21;q21) product in MALT lymphoma is linked to deregulated ubiquitin ligase activity. *Cancer Cell* 2005; **7**:425-431.
106 Wu C, Ashwell JD. NEMO recognition of ubiquitinated Bcl10 is required for T cell receptor-mediated NF-κB activation. *Proc Natl Acad Sci USA* 2008; **105**:3023-3028.
107 Ferch U, Buschenfelde CMZ, Gewies A, *et al*. MALT1 directs B cell receptor-induced canonical nuclear factor-κB signaling selectively to the c-Rel subunit. *Nat Immunol* 2007; **8**:984-991.
108 Dong W, Liu Y, Peng J, *et al*. The IRAK-1-BCL10-MALT1-TRAF6-TAK1 cascade mediates signaling to NF-κB from Toll-like receptor 4. *J Biol Chem* 2006; **281**:26029-26040.
109 Gross O, Gewies A, Finger K, *et al*. Card9 controls a non-TLR signaling pathway for innate anti-fungal immunity. *Nature* 2006; **442**:651-656.
110 Gross O, Grupp C, Steinberg C, *et al*. Multiple ITAM-coupled NK cell receptors engage the Bcl10/Malt1 complex via Carma1 for NF-κB and MAPK activation to selectively control cytokine production. *Blood* 2008; **112**:2421-2428.
111 Klemm S, Gutermuth J, Hültner L, *et al*. The Bcl10-Malt1 complex segregates Fc epsilon RI-mediated nuclear factor κB activation and cytokine production from mast cell degranulation. *J Exp Med* 2006; **203**:337-347.
112 Klemm S, Zimmermann S, Peschel C, Mak TW, Ruland J. Bcl10 and Malt1 control lysophosphatidic acid-induced NF-κB activation and cytokine production. *Proc Natl Acad Sci USA* 2007; **104**:134-138.
113 Martin D, Galisteo R, Gutkind JS. CXCL8/IL8 stimulates vascular endothelial growth factor (VEGF) expression and the autocrine activation of VEGFR2 in endothelial cells by activating NFκB through the CBM (Carma3/Bcl10/Malt1) complex. *J Biol Chem* 2009; **284**:6038-6042.
114 McAllister-Lucas LM, Ruland J, Siu K, *et al*. CARMA3/Bcl10/MALT1-dependent NF-κB activation mediates angiotensin II-responsive inflammatory signaling in nonimmune cells. *Proc Natl Acad Sci USA* 2007; **104**:139-144.
115 Tusche MW, Ward LA, Vu F, *et al*. Differential requirement of MALT1 for BAFF-induced outcomes in B cell subsets. *J Exp Med* 2009; **206**:2671-2683.
116 Rehman AO, Wang C. CXCL12/SDF-1α activates NF-κB and promotes oral cancer invasion through the Carma3/Bcl10/Malt1 complex. *Int J Oral Sci* 2009; **1**:105-118.
117 Hara H, Saito T. CARD9 versus CARMA1 in innate and adaptive immunity. *Trends Immunol* 2009; **30**:234-242.
118 Uren AG, O'Rourke K, Aravind LA, *et al*. Identification of paracaspases and metacaspases: two ancient families of caspase-like proteins, one of which plays a key role in MALT lymphoma. *Mol Cell* 2000; **6**:961-967.
119 Rebeaud F, Hailfinger S, Posevitz-Fejfar A, *et al*. The proteolytic activity of the paracaspase MALT1 is key in T cell activation. *Nat Immunol* 2008; **9**:272-281.
120 Coornaert B, Baens M, Heyninck K, *et al*. T cell antigen receptor stimulation induces MALT1 paracaspase-mediated cleavage of the NF-κB inhibitor A20. *Nat Immunol* 2008; **9**:263-271.
121 Coornaert B, Carpentier I, Beyaert R. A20: central gatekeeper in inflammation and immunity. *J Biol Chem* 2009; **284**:8217-8221.
122 Düwel M, Welteke V, Oeckinghaus A, *et al*. A20 negatively regulates T cell receptor signaling to NF-κB by cleaving Malt1 ubiquitin chains. *J Immunol* 2009; **182**:7718-7728.
123 Malinverni C, Unterreiner A, Staal J, *et al*. Cleavage by MALT1 induces cytosolic release of A20. *Biochem Biophys Res Commun* 2010; **400**:543-547.
124 Ferch U, Kloo B, Gewies A, *et al*. Inhibition of MALT1 protease activity is selectively toxic for activated B cell-like diffuse large B cell lymphoma cells. *J Exp Med* 2009; **206**:2313-2320.
125 Hailfinger S, Lenz G, Ngo V, *et al*. Essential role of MALT1 protease activity in activated B cell-like diffuse large B-cell lymphoma. *Proc Natl Acad Sci USA* 2009; **106**:19946-19951.