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Review

Non-apoptotic functions of caspase-8

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ABSTRACT

Caspases are a family of aspartate-specific cysteine proteases that have been well characterized for their function in apoptosis signaling. Caspase-8 is implicated as an initiator caspase in death receptor-induced signaling to apoptosis and has been studied most extensively for its role in CD95-induced cell death. CD95 stimulation induces the binding of caspase-8 to a death-inducing signaling complex, leading to its autocatalytic cleavage and the formation of a caspase-8 homodimer, which is subsequently released into the cytosol where it further mediates the apoptotic signaling cascade. Over the past few years, however, several non-apoptotic functions for caspase-8 have been described, indicating that this protease plays a much more diverse role than previously assumed. Here we review the role of caspase-8 in embryonic development, monocyte differentiation, T and B cell proliferation, and the activation of NF-κB.

Contents

1. Introduction

Caspases belong to a family of conserved aspartate-specific cysteine proteases with functions in apoptosis and immune signaling. According to the hierarchy of activation, they can be subdivided in two major groups. A first group is called the initiator caspases, since they are the first to be activated upon a death- or inflammation-inducing signal. Initiator caspases are synthesized as zymogens, containing a large N-terminal prodomain, such as the caspase recruitment domain (CARD; in

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case of caspase-1, -2, -4, -5, -9, -11 and -12) or the death effector domain (DED; in case of caspase-8 and -10), and a C-terminal catalytic subunit composed of a small and a large domain, separated by a small linker region [1,2]. Upon triggering of cells with certain apoptotic or inflammatory danger signals, initiator caspases are recruited to large multiprotein complexes by virtue of their prodomains. Dimerization of the initiator caspases and the subsequent conformational changes at the receptor complex results in the formation of catalytic active caspase dimers [3]. Well defined caspase activating complexes include the apoptosome (activating caspase-9) [4], the inflammasome (activating caspase-1) [5], the piddosome (activating caspase-2) [6], and the death-inducing signaling complex (DISC; activating caspase-8) [7]. A second group of caspases known as the executioner or effector caspases (caspase-3, -6, -7 and -14), contain only a small N-terminal prodomain. They exist as preformed homodimers in the cytoplasm and are activated through proteolytic maturation by the initiator caspases, rendering them catalytically active. Once activated, these caspases cleave a wide variety of cellular substrates, eventually leading to apoptosis of the cell [2].

Caspase-8 has been extensively studied as the initiator caspase of the extrinsic apoptosis pathway, which is triggered by several death receptors belonging to the TNF-receptor type 1 superfamily containing TNF receptor I (TNFRI), CD95, TRAIL receptor (DR4), DR3 and DR6. Oligomerization of CD95 upon binding of its ligand or agonistic antibodies leads to recruitment of the Fas-associated death domain (FADD) protein, caspase-8, caspase-10 (in case of humans) and cellular FLICE inhibitory protein ($cFLIP_L$), forming the DISC [8]. Within this complex caspase-8 dimerizes into its catalytically active form. Subsequently, the N-terminal DED is cleaved off, allowing the activated caspase to be released in the cytosol where it can target downstream effector caspases such as caspase-3 and -7. Caspase-10 is only present in humans and has no known functional homolog in mice [9]. Apparently caspase-8 and -10 have partially redundant cellular functions during apoptosis [10]. $cFLIP_L$, a caspase-8 like protein that contains two DEDs and a pseudo-caspase-8 domain containing an inactive catalytic site, is also found at the DISC. When $cFLIP_L$ is present in low concentrations it acts as a positive regulator of caspase-8 activation. However, increasing amounts of $cFLIP_L$ inhibit caspase-8 activation, probably by replacing caspase-8 at the DISC and thus dampening apoptosis [11].

As a way of amplifying the apoptotic signal, caspase-8 can also activate the intrinsic apoptotic pathway through the cleavage of BH3 interacting domain death agonist (BID), a B-cell lymphoma 2 (BCL-2)-homology domain 3 only (BH3-only) protein. BID is a specific proximal substrate for caspase-8 and once cleaved it translocates from the cytosol to the outer mitochondrial membrane, where it interacts with BCL-2 associated protein X (BAX) and BCL-2 antagonist/killer (BAK), allowing BAX and BAK to oligomerize. This triggers the release of cytochrome *c* in the cytoplasm, thereby activating the Apaf-1/caspase-9 apoptosome [12].

Surprisingly, caspase-8 is not always involved in cell death signaling. In the last few years substantial evidence has been built up regarding non-apoptotic functions of caspase-8, involving embryonic development, NF-κB activation, T and B cell proliferation and macrophage differentiation (Table 1). In this review we focus on the engagement of caspase-8 in these non-apoptotic signaling pathways.

2. A role for caspase-8 in embryonic development

Targeted disruption of the mouse *caspase-8* gene was proven to be embryonic lethal, which is caused by major developmental defects at day E11.5 of gestation. At this point in development, caspase-8 knockout mice suffer from abnormalities in yolk sac

Table 1 – Overview of phenotypes observed in caspase-8 knockout mouse models

Function	Studied model(s)	Phenotype	References
Embryonic development	Full $casp8^{-/-}$; Tie-Cre $casp\text{-}8^{fl/-}$	Embryonical death around day E11.5, hyperemia, liver erythrocytosis, impaired heart development, defects in neural tube	[13–15]
Hematopoietic precursor development	Mx-Cre $casp8^{fl/-}$	Decrease in functionality of $casp8^{-/-}$ bone marrow progenitor cells	[15]
Monocyte differentiation	LysM-Cre $casp8^{fl/-}$	Impaired monocyte differentiation into macrophages, decreased survival of monocytes upon M-CSF treatment	[15]
T cell activation and proliferation	Lck-Cre $casp8^{fl/-}$	Reduced proliferation and increased cell death upon TCR triggering, impaired NF-κB activation, increased susceptibility to infection with Lymphocytic Choriomeningitis Virus	[35]
B cell proliferation	CD19-Cre $casp8^{fl/-}$	Impaired proliferation, increased cell death and reduced NF-κB activation upon TLR-3 and TLR-4 stimulation	[43,63]
Liver regeneration, cellular response to infection	Alb-Cre $casp8^{fl/-}$	Attenuated early liver regeneration, increased susceptibilty to infection with *Listeria monocytogenes*	[78]

vasculature formation and hyperemia in most major blood vessels and many organs, accompanied by extensive erythrocytosis in the liver and impaired heart muscle development [13]. In another study, a partial knockout of caspase-8 was generated, expressing only the DED domain of caspase-8, thus generating a specific dominant negative mutant, allowing to interfere also with a possible murine analog of caspase-10 [14]. Besides a harmful effect on the development of the neural tube, possibly overlooked by Varfolomeev et al., the phenotype observed in these mice was much alike that of the complete caspase-8 knockout. Strikingly, the neural and heart defects could be rescued by *ex vivo* culturing of the embryo at day E10.5–E11.5, indicating that the defects seen in heart and neural tube are caused by secondary effects, most likely due to the impairment of angiogenesis of the yolk sac. Indeed, cell-specific deletion of caspase-8 in endothelial cells, using mice that express Cre recombinase under control of the endothelium-specific Tie1 promoter died during embryogenesis, suffering from the same gross abnormalities seen in the full caspase-8 knockout embryos [15]. This indicates that caspase-8 plays a crucial non-apoptotic role during the development of the yolk sac vasculature.

Interestingly, mice deficient in the caspase-8 interacting proteins FADD [16] or $cFLIP_L$ [17] display a similar phenotype as the caspase-8 knockout mice. However, neither CD95-, TNFR1-, DR3-, DR6-, nor TRAIL-deficient embryos show a similar phenotype and all mice are viable [18–23], arguing for a death receptor independent role for the FADD/caspase-8/$cFLIP_L$ signaling axis during embryogenesis.

3. Caspase-8 is required for monocyte differentiation

Deletion of the *caspase-8* gene in the myeloid cell compartment using the Lysozyme M-Cre mouse line revealed a critical role for caspase-8 during monocyte differentiation into macrophages. When cultured in the presence of macrophage colony-stimulating factor (M-SCF), caspase-8 deficient monocytic bone marrow precursor cells fail to differentiate into macrophages, while the differentiation process into dendritic cells and granulocytes remains unaffected [15]. These findings are consistent with previous data showing that human peripheral blood monocytes stimulated with M-CSF exhibit limited caspase-3, -8 and -9 activation, which does not lead to apoptosis and could not be observed when monocytes were prone towards dendritic cell differentiation. In addition, macrophage differentiation could be blocked by several caspase inhibitors such as baculovirus p35, cowpox virus CrmA or the pan-caspase inhibitor z-VAD-fmk [24]. The differentiation process from monocytes into macrophages requires changes in the cellular state, such as cytoskeleton rearrangements, changes in cell adhesion and differential transcriptional regulation. At least in part, this process seems to be regulated through cleavage of specific proteins by caspases, without inducing apoptotic cell death. Thus far, an apical role has been assigned to caspase-8 during the differentiation process: caspase-8 activation induces the activation of executioner caspases like caspase-3 and -9, allowing a large number of substrates to be processed. One of the first substrates that is cleaved by caspase-8 upon M-CSF stimulation is receptor interacting protein 1 (RIP1). Processing of RIP1 by caspase-8 prevents the sustained activation of NF-κB, which favors the proper differentiation into macrophages [25]. In an attempt to identify specific caspase substrates implicated in monocyte differentiation, Solary and colleagues used mass spectrometry to analyze differential cleavage products between control and p35 expressing U937 monocytic cells treated with phorbol ester, allowing them to differentiate into macrophages [26]. A number of substrates involved in cytoskeletal regulation were identified such as α-tubulin, β-actin and vinculin-α. Other substrates include proteins involved in transcriptional regulation (hnRNPs) and apoptosis (PAK-2, PAI-2). Interestingly, poly ADP-ribose polymerase and lamin B, both targets of the proteolytic activity of caspase-3 during apoptosis, are protected from processing during monocyte differentiation, suggesting that selective processing of substrates is an important regulation mechanism allowing the cell to discriminate between differentiation and apoptosis [27]. Since caspase-8 might prove to be an interesting target in malignant human conditions affecting the monocyte population, such as chronic myelomonocytic leukemia, it will be of high interest to further address the molecular mechanism leading to caspase-8 activation after M-CSF stimulation of monocytes and to identity those substrates whose cleavage contributes to the monocyte differentiation process.

4. Caspase-8: a key regulator of T cell activation

Individuals that carry homozygous mutations (R248W) in *caspase-8* suffer from autoimmune lymphoproliferative syndrome (ALPS)-like symptoms. ALPS is a disease marked by lymphoadenopathy, splenomegaly and autoimmunity. This is caused by defective lymphocyte apoptosis during negative selection of autoreactive double positive ($CD4^+/CD8^+$) T cells and failure to clear peripheral T cells by a process known as activation-induced cell death. Previously, heterozygous mutations in CD95, CD95 ligand and caspase-10 have also been linked to this condition [28–31]. Strikingly, besides partial defects in lymphocyte apoptosis, caspase-8 deficient patients also show a clear defect in the activation of their T and B lymphocytes and NK cells, accompanied by recurrent sinopulmonary herpes simplex virus infections and poor responses to immunization [32]. Unlike the phenotype seen in caspase-8 deficient mice, caspase-8 deficient humans have no major developmental defects and the phenotype seems to be more restricted to defects in their immune system. An explanation for the difference between both species might be that residual caspase-8 activity in the human patients rescues the developmental phenotype, but not the lymphoproliferative phenotype. Another hypothesis is that capase-10, the closest paralog of caspase-8 in humans that does not have an ortholog in mice might compensate for the loss of caspase-8 function.

CD3/CD28-induced proliferation of human T cells was shown to be accompanied by rapid cleavage of caspase-8, but not of caspase-3, and this in the absence of apoptosis. Blocking caspase-8 activation at early time points during T cell

activation with the broad spectrum caspase inhibitor z-VAD-fmk or its more specific inhibitor z-IETD-fmk decreased T cell proliferation and interleukin-2 (IL-2) production, indicating that caspase-8 plays an unexpected and non-redundant role in the proliferative response of these cells [33,34]. This was further demonstrated *in vivo* by deleting caspase-8 specifically in T cells using the Lck-Cre or CD4-Cre lines; caspase-8 deficient T cells failed to proliferate both *in vitro* and *in vivo* after T cell receptor (TCR) stimulation. Also, a significant depletion of particularly $CD8^+$ peripheral T cells was observed in the secondary lymphoid tissues, indicating an essential role for caspase-8 in peripheral T cell homeostasis [35]. Not only caspase-8, but also FADD and $cFLIP_L$ seem to play important functions in T cell proliferation. Independent experiments using transgenic mice that express a dominant negative form of FADD (containing only its death domain) under the control of the T cell-specific Lck promoter, thus inhibiting further downstream signaling through caspase-8, show that FADD is an important mediator of immature thymocyte development at the level of the DN3 stage [36,37]. Reconstitution of RAG-1 deficient blastocysts with FADD deficient embryonic stem cells, resulting in chimaeric mice that only develop FADD deficient lymphocytes, led to the same observations [16]. However, a more recent study showed that specific deletion of FADD in the T cell compartment does not affect early thymocyte development although peripheral T cell homeostasis and proliferation was severely impaired [38]. These data correlate with the data obtained from T cell-specific conditional caspase-8 knockout studies showing that early T cell development is relatively normal [35]. Using similar approaches, also $cFLIP_L$ was shown to play a crucial role in T cell homeostasis and TCR-induced proliferation [39,40]. These observations and the fact that no known death receptors or other caspases have been implicated in the same way as FADD, caspase-8 and $cFLIP_L$ in T cell survival and proliferation, indicate that a DISC-like complex is operating in a death receptor independent way in the T cell compartment.

Several models have been proposed to explain the necessity of caspase-8 in T cell development. Su et al. published a breakthrough article in which they describe that caspase-8 is an essential modulator of NF-κB activation, which is an essential transcription factor for proper T cell activation [41]. The authors describe that NF-κB activation upon TCR stimulation is impaired in T cells from caspase-8 deficient patients, caspase-8 deficient Jurkat cells and caspase-8 deficient murine T cells. By immunoprecipitating specifically activated caspase-8 using biotinylated z-VAD-fmk it was demonstrated that only a minor fraction (10–15%) of the total caspase-8 pool is activated upon TCR stimulation. Activated caspase-8 is able to associate with the T cell signaling molecules B cell leukaemia/lymphoma 10 (Bcl10) and the paracaspase mucosa associated lymphoid tissue lymphoma translocation gene 1 (MALT1). Recruitment of caspase-8 to these molecules allows the NF-κB activating IκB kinase holoenzyme to be sequestered to the TCR signaling platform, resulting in NF-κB activation [41]. Using the same immunoprecipitation technique the researchers later found that also the ubiquitin ligase TNF receptor-associated factor 6 (TRAF6) binds to active caspase-8 upon TCR stimulation and facilitates its movement into lipid rafts [42]. NF-κB signaling is not only impaired in caspase-8 deficient T cells, but also human B cells and NK cells are affected in their NF-κB signaling, as is illustrated by the finding that B and NK cells from caspase-8 deficient patients fail to translocate p65 to the nucleus upon B cell receptor (BCR) and FcγRIII stimulation, respectively [41]. In contrast, BCR-induced NF-κB activation is unaffected in caspase-8 deficient or FADD deficient mice, suggesting that caspase-8 plays a unique role in human B and NK cell activation, but is dispensable in murine B cells [43,44].

Sequestering activated caspase-8 from the cytoplasm to lipid rafts following T cell stimulation might be an important regulatory mechanism by which a micro-environment is formed where NF-κB activating substrates are cleaved and apoptosis inducing substrates remain unprocessed. One putative substrate for caspase-8 at the TCR complex is the caspase-8 homologue $cFLIP_L$ because this protein has been described before to activate NF-κB and Erk MAP kinase in T cells through binding of TRAF1, TRAF2, RIP1 and Raf-1. Moreover, upon TCR stimulation $cFLIP_L$ was shown to be cleaved in a caspase-8 dependent manner into a $p43^{FLIP}$ isoform, which has a greater capacity than its uncleaved counterpart to recruit RIP1 and TRAF2, resulting in increased NF-κB activation and IL-2 production [45,46]. Furthermore, Jurkat cells overexpressing $cFLIP_L$ or T cells from $cFLIP_L$ transgenic mice display augmented IL-2 production in response to CD3/CD28 stimulation [47]. Also, $CD8^+$ T cells from $cFLIP_L$ transgenic mice show an increased NF-κB activity and a decreased activation threshold, manifested by an increased proliferation to low dose antigens or low affinity antigens [48]. Recently it was shown that $cFLIP_L$ and caspase-8 are recruited simultaneously to lipid rafts and $cFLIP_L$ is cleaved into the p43 form shortly after CD3/CD28 stimulation [49]. The active role of caspase-8 and $cFLIP_L$ in TCR-induced NF-κB activation is further demonstrated by transgenic T cells expressing a shorter form of cFLIP ($cFLIP_S$) lacking the C-terminal caspase-8 like domain and acting in a dominant negative way on caspase-8 activation. These cells show impaired NF-κB activation due to reduced Bcl10 and MALT1 recruitment to the TCR signaling complex [50]. Apart from its contribution to NF-κB activation, the recruitment of caspase-8 to the TCR signaling complex might also be a way of regulating T cell apoptosis upon repeated stimulation with its antigen, a process known as activation-induced cell death. Prolonged caspase-8 activation due to TCR signaling might shift the balance from cell survival towards apoptosis.

Besides the above described effects on NF-κB signaling, an increase of the G1 T cell subpopulation was observed upon TCR triggering of caspase-8 deficient T cells, probably caused by increased cell death among the proliferating cells or alternatively by a defect in cell cycle progression [35]. Interestingly, a similar phenotype was observed in T cells from T cell-specific FADD dominant negative transgenic mice and FADD deficient mice [38,44,51,52]. By using caspase-8 and FADD deficient T cells, as well as transgenic T cells expressing the viral caspase-8 inhibitor vFLIP, it was shown that caspase-8 and FADD are required for the prolonged S6 kinase 1 activity seen after long-term IL-2 stimulation. This sustained kinase activity is essential for the complete phosphorylation of the ribosomal protein S6 on Ser^{240} and Ser^{244}, and possibly other substrates that contribute to the S-phase entry during the cell

cycle [53]. However, the exact molecular mechanism by which caspase-8 contributes to cell cycle progression remains obscure and nothing is known about the identity of any of its substrates that might regulate the transition form G1 to S phase. Alternatively, the impairment of T cell proliferation of caspase-8 deficient T cells can also be explained in terms of increased cell death; clonal expansion of T cells is a tightly regulated process that depends on the delicate interplay between cell survival and apoptosis. By removing caspase-8 from the cellular compartment this balance might be disturbed. One would expect that deletion of caspase-8 would benefit T cell survival. However, the experiments with caspase-8 knockout T cells reveal an opposite effect. At least in part, this phenotype could be caused by the diminished NF-κB activation after TCR stimulation, since NF-κB is a known antagonist of apoptosis. However such severe proliferative defects could not all be contributed to impaired NF-κB activation. Several reports state that caspase-8 removal causes a shift from an apoptotic towards a necrotic like autophagic cell death [54,55]. It is not unlikely that the impaired cell cycle progression seen in caspase-8 deficient T cells reflects increased cell death of T cells during clonal expansion and is rather a consequential than a causal phenotype.

5. A role for caspase-8 in NF-κB signaling in innate immunity

Toll-like receptors (TLRs) are important regulators of the innate immune system that recognize specific microbial components and trigger the induction of pro-inflammatory cytokines, chemokines and costimulatory molecules mounting a protective immune response [56]. TLR3 and TLR4, which recognize viral double stranded RNA and bacterial lipopolysaccharide (LPS), respectively, use the adaptor protein TRIF (TIR domain containing adaptor inducing interferon-β) to activate NF-κB and interferon regulatory factor (IRF) (Fig. 1), which control the expression of proinflammatory cytokines and type-I IFN genes, respectively [57]. In addition, TLR4 can also initiate NF-κB activation in a TRIF-independent manner via the adaptor protein MyD88 (myeloid differentiation primary response gene 88). Besides inducing a proinflammatory response, TRIF is also a known inducer of programmed cell death for which it requires the cooperative binding with RIP1, FADD and the activation of caspase-8 [58]. This TRIF-dependent pathway has been linked to early apoptosis of macrophages and dendritic cells after bacterial infection mediated by TLR4 and is strictly dependent on caspase-8 activation [59,60]. By inhibiting translation or NF-κB-dependent gene expression, cells can be further sensitized to TLR3- or TLR4-induced apoptosis [61,62]. Apart from initiating apoptosis upon TLR3 and TLR4 stimulation, caspase-8 has also been reported to contribute to the TLR-induced NF-κB activation cascade. Caspase-8 deficient B cells display delayed nuclear translocation of NF-κB after TLR3 and TLR4 stimulation, but not after TLR9 stimulation, which is entirely dependent on the MyD88 adaptor protein [41,63]. Co-immunoprecipitation experiments in both B and MEF cells revealed that caspase-8 associates transiently with the IKK complex shortly after TLR4 stimulation. If caspase-8 has a scaffolding function bringing the IKK complex in proximity with other signaling molecules rather than playing an active role in NF-κB signaling remains to be determined. In addition to defects in NF-κB signaling, caspase-8 and also FADD deficient B cells fail to proliferate in response to double stranded RNA and LPS as a consequence of an increase in cell death [63,64]. These survival defects of caspase-8 deficient B cells during TLR3- and TLR4-induced proliferation shows striking similarities to the impaired proliferation observed in caspase-8 deficient T cells. Why caspase-8 is not required for BCR-induced proliferation (at least in murine B cells) is still an unsolved matter.

TLR3 and TLR4 are not the only innate immune receptors that have been reported to require caspase-8 for NF-κB activation. Two other type I IFN inducing receptors, retinoid acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA5), have been reported to induce caspase-8 processing and appear to depend on caspase-8 for NF-κB induction [65]. RIG-I and MDA5 are cytosolic sensors of viral RNA products, recognizing 5′ triphosphorylated RNA [66,67] and dsRNA [68], respectively. Upon ligand binding, RIG-I and MDA5 associate through CARD homotypic interactions with the adaptor protein virus-induced signaling adapter (VISA), also known as Cardif, MAVS or IPS1 [69], which subsequently binds to TNFRSF1A-associated via death domain (TRADD), where the signal bifurcates into a TRAF3-dependent arm for the activation of IRF and a RIP1/FADD-dependent arm for the induction of NF-κB [70]. With the help of siRNA-mediated knockdown of caspase-8 and the use of caspase-8 deficient MEF cells it was shown that caspase-8 activation contributes to NF-κB activation, but not IRF activation, in response to cytosolic dsRNA [65]. Similarly, FADD was shown to be involved in the activation of NF-κB upon intracellular dsRNA signaling [71], implicating that FADD and caspase-8 operate in a signaling axis skewed towards NF-κB activation.

TLR3, TLR4, RIG-I and MDA5 all share a common signaling pathway involving RIP1, FADD and caspase-8 to activate NF-κB. Interestingly, this pathway is very well conserved during evolution as it shows striking similarities with the *Drosophila* immune deficiency (IMD) signaling cascade (Fig. 1) [72]. *Drosophila* elicits an immune response versus Gram-negative bacteria through recognition of peptidoglycan by the transmembrane peptidoglycan recognition protein (PGRP-LC), leading to activation of Relish, the fly homologue of the mammalian p100 and p105 NF-κB transcription factor precursors [73]. Upon ligand binding, the PGRP-LC receptor associates with IMD (the fly homologue of RIP1), leading to the activation of TAK1, which phosphorylates the IKKβ-like protein IRD5 (immune-response deficient 5). Phosphorylation of Relish by IRD5 is thought to target Relish for partial proteasomal degradation, similar to what happens with the mammalian homologues p100 and p105, where the inhibitory IκB-like part is degraded and the residual transcriptional active protein shuttles to the nucleus [74]. However, phosphorylation-dependent proteasomal degradation is not sufficient for complete activation of Relish. Through a second signaling arm departing from IMD, the fly caspase-8 homo-

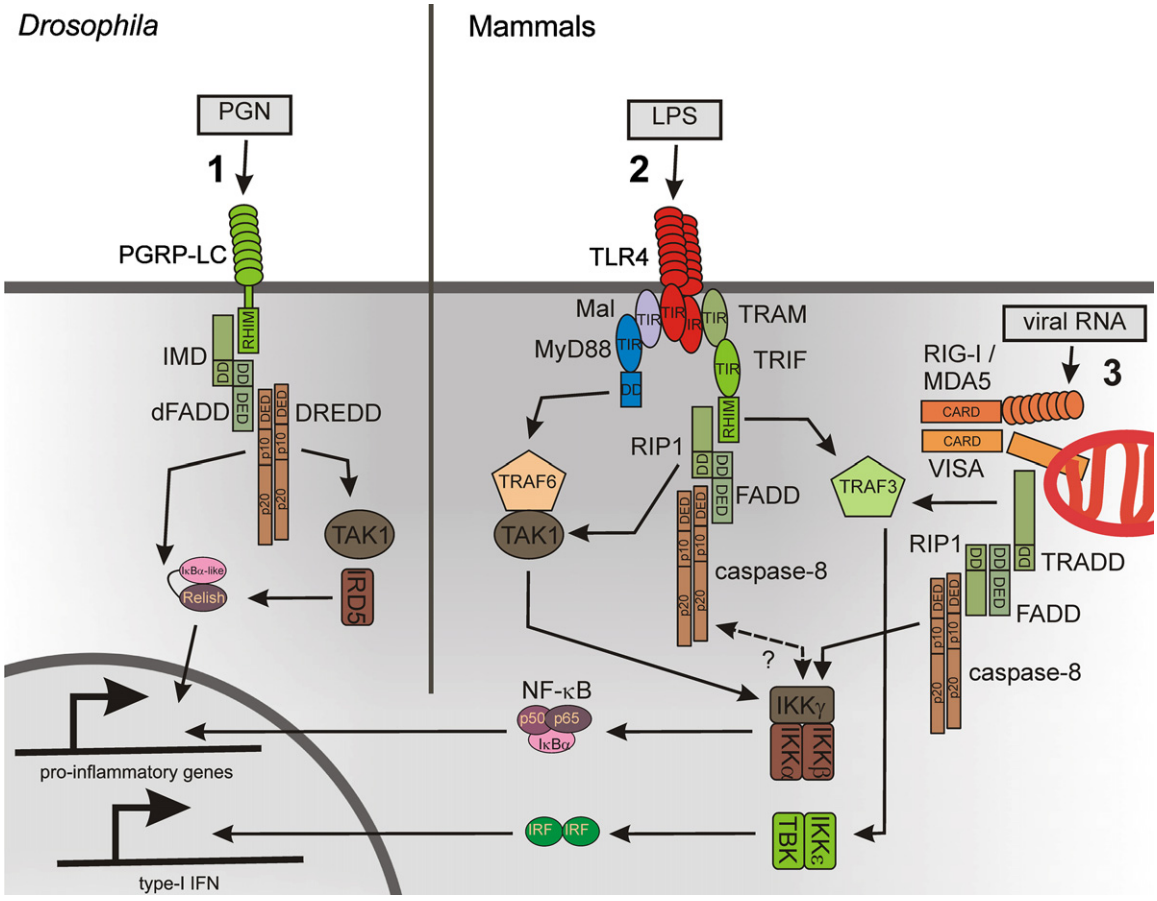

Fig. 1 – Similarity between the *Drosophila* IMD pathway and the mammalian RIP1 pathway leading to NF-κB activation. (1) IMD pathway: peptidoglycan (PGN) from the bacterial cell wall binds to its receptor PGRP-LC on the cell surface. Subsequently, IMD, dFADD and DREDD are recruited to the receptor, resulting in the activation of DREDD, which cleaves off the IκBα-like subunit of Relish. Another arm, departing from IMD activates the kinase TAK1, which phosphorylates and activates the IKKβ-like protein IRD5 in a DREDD-dependent manner. IRD5 in turn phosphorylates the IκBα-like subunit of Relish, targeting it for proteasomal degradation, allowing the transcriptionally active Relish subunit to translocate to the nucleus. (2) TLR4-dependent RIP1 pathway: upon LPS binding, both the MyD88 and TRIF adaptor proteins translocate to the receptor. Subsequently, RIP1 binds to TRIF and activates TAK1, leading to the activation of the NF-κB activating IKK complex. Caspase-8 might act as a scaffolding protein by bringing the IKK-complex in close proximity to its activator TAK1. Alternatively, NF-κB can also be activated by the MyD88-dependent pathway in a TRAF6/TAK1-dependent manner. TRIF also induces the activation of TRAF3 which in turn activates the IRF activation complex, consisting of either TBK or IKKε. Activated IRF translocates into the nucleus and induces type-I IFN induction. (3) RIG-I/MDA5-dependent RIP1 pathway: binding of viral RNA to either RIG-I or MDA5 allows these receptors to bind to the VISA adaptor protein located at the mitochondrion. In a TRADD/FADD/RIP1/caspase-8-dependent manner this leads to the activation of NF-κB. TRAF3 binding to VISA with the help of TRADD leads to the activation of the IRF activating TBK or IKKε kinases, eventually leading to type-I IFN induction. *Abbreviations*: RIP homology interaction motif (RHIM); death domain (DD); death effector domain (DED); small and large catalytic subunits of caspase-8 (p10/p20); Toll/Interleukin-1 receptor domain (TIR); caspase recruitment domain (CARD); inhibitor of NF-κB (IκB); interferon regulatory factor (IRF).

logue death-related ced-3/Nedd2 like protein (DREDD) is activated in a dFADD (*Drosophila* FADD)-dependent manner and proteolytically cleaves Relish, which separates the transcriptional active subunit and the IκB homology domain that is targeted for further proteasomal degradation [75]. With the notable exception of TRAIL receptor stimulation, where caspase-8 mediated cleavage of IκBα contributes to sustained NF-κB activation [76], IκB processing by caspase-8 has not been observed in mammals. Alternatively, caspase-8 might operate at a different level in the NF-κB signaling cascade. As described before, caspase-8 appears to associate shortly after TCR as well as TLR4 stimulation with the IKK complex. Strikingly, apart from cleaving Relish, DREDD is also required for the activation of the IKK complex in *Drosophila* [77], supporting the hypothesis that caspase-8 exerts a bridging function between the IKK complex and upstream signaling proteins.

Clearly, the molecular mechanisms by which caspase-8 attributes to NF-κB signaling is still an open question.

Nothing is known about the identity of putative caspase-8 substrates involved in NF-κB signaling. Interestingly, NF-κB activation through caspase-8 may not always require its enzymatic activity. For TCR signaling it is known that caspase-8 catalytic activity at least contributes to NF-κB activation, but NF-κB activation after TLR and RIG-I/MDA5 stimulation might be independent of caspase-8 enzymatic activity and only involve a scaffolding function of caspase-8. To fully understand the contribution of caspase-8 at the level of NF-κB signaling, questions like these will have to be addressed in the future.

6. Conclusions

It is clear that caspase-8 is an important regulator of several non-apoptotic cellular functions, such as proliferation, differentiation and NF-κB activation. Caspase-8 activation during these processes has to be tightly regulated, since enhanced caspase-8 activation irreversibly tips the balance from survival to apoptotic cell death. Intriguingly, all the non-apoptotic processes implicating caspase-8 are associated with a rather limited caspase-8 activation. 'Hyperactivation' of caspase-8 under non-apoptotic circumstances might be dampened by the selective upregulation of anti-apoptotic genes such as $cFLIP_L$. Selective processing of substrates could also be a mechanism to avoid cell death. Cleaving specific targets might generate protein fragments that provide protective effects towards apoptosis. How caspase-8 and possibly also other caspases decide in their substrate specificity remains poorly understood. One possibility is that subcellular localization plays a decisive role; for example during T cell activation, caspase-8 is recruited to lipid rafts, allowing it to interact with different substrates. Apart from a potential role of limited caspase-8 activation and subcellular localization in determining selectivity in substrate processing and preventing cell death, caspase-8 can also initiate signals that protect the cell from autophagic or necrotic cell death. The underlying molecular mechanism is poorly understood, but preventing necrotic cell death might be an important survival mechanism, especially in highly proliferative processes like clonal B and T cell expansion. Clearly, further research is needed to understand the exact molecular mechanism involved in each of these processes. Interfering with caspase-8 activity might eventually prove to be a interesting therapeutic approach to treat several inflammatory disorders involving cells of the innate or adaptive immune system.

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