Cell Research (2011) 21:1383-1385.

www.nature.com/cr

RESEARCH HIGHLIGHT

FADD: an endogenous inhibitor of RIP3-driven regulated necrosis

Lorenzo Galluzzi[1, 2, 3], Oliver Kepp[1, 2, 3], Guido Kroemer[1, 4, 5, 6, 7]

[1]INSERM, U848, Institut Gustave Roussy, PR1, 39 rue Camille Desmoulins, F-94805 Villejuif, France; [2]Institut Gustave Roussy, Villejuif, France; [3]Université Paris-Sud/Paris XI, Le Kremlin-Bicêtre, Paris, France; [4]Metabolomics Platform, Institut Gustave Roussy, Villejuif, France; [5]Centre de Recherche des Cordeliers, Paris, France; [6]Pôle de Biologie, Hôpital Européen Georges Pompidou, AP-HP, Paris, France; [7]Université Paris Descartes, Sorbonne Paris Cité, Paris, France
Cell Research (2011) **21**:1383-1385. doi:10.1038/cr.2011.147; published online 6 September 2011

Thanks to the work of multiple laboratories worldwide, the classical dichotomy postulating that apoptosis and necrosis would constitute diametrically opposed cell death subroutines has recently been dismissed [1]. Thus, approximately in the same year when immunogenic instances of apoptosis were first characterized [2], the traditional belief that necrosis would represent a merely accidental, unregulated cell death modality has been definitely abandoned [3]. This has fueled an intense wave of research, advancing our understanding of the molecular mechanisms and pathophysiological implications of regulated necrosis [4, 5]. The first and perhaps best-characterized signaling cascade that leads to regulated necrosis involves death receptor signaling in the context of apoptosis incompetence (e.g., due to chemical or genetic inhibition of caspase-8), leading to the activation of receptor-interacting protein kinases 1 and 3 (RIP1 and RIP3) [5, 6]. The molecular mechanisms that execute regulated necrosis downstream of RIP1/RIP3 activation have not yet been entirely elucidated, though it has been suggested that RIP3 might trigger a bioenergetic catastrophe by hyperactivating several enzymes involved in glycogenolysis and glutaminolysis [5]. Irrespective of these uncertainties, it is now clear that the ligation of some death receptors, in particular tumor necrosis factor α (TNF) receptor 1 (TNFR), can have at least three well distinct biological outcomes: (i) activation of NF-κB-dependent pro-survival signaling, (ii) caspase-8 processing and execution of extrinsic apoptosis, or (iii) activation of the RIP1/RIP3 complex and induction of regulated necrosis [5, 7]. The molecular determinants that regulate this complex signaling hub have only recently begun to emerge. In a recent paper published by *Nature*, Welz *et al.* demonstrate that FADD, a death domain-containing cytoplasmic adaptor protein that is required for the execution of extrinsic apoptosis [8], operates as an endogenous inhibitor of RIP3-mediated regulated necrosis in the intestinal epithelium [9].

As *Fadd*$^{-/-}$ mice do not survive beyond day 11.5 of embryogenesis [8, 10], to investigate the role of FADD in the intestinal epithelium, Welz and colleagues generated mice with an intestinal epithelial cell (IEC)-specific deletion of *Fadd* (which they dubbed FADD$^{IEC\text{-}KO}$ mice). FADD$^{IEC\text{-}KO}$ mice were born normally but rapidly developed a severe and spontaneous intestinal phenotype resulting in the death of approximately 50% of the animals before weaning. Surviving animals displayed reduced body weight, diarrhea, as well as signs of severe colitis such as immune cell infiltration of the intestinal mucosa and local cytokine production. The immune infiltrate included F4/80$^+$ and Gr-1$^+$ myeloid cells as well as – especially in older animals – B and T lymphocytes. Still, diarrhea and colitis were observed when FADD$^{IEC\text{-}KO}$ mice were crossed with *Rag1*$^{-/-}$ mice, proving that B and T cells are not essential for intestinal inflammation in this model [9].

Driven by the fact that dying *Fadd*$^{-/-}$ IECs failed to stain positively for caspase-3 activation, exhibited ultrastructural signs of necrosis and expressed higher levels of RIP3 than their wild-type counterparts, Welz *et al.* decided to introduce the IEC-specific deletion of *Fadd* on genetic backgrounds deficient for central modulators of regulated necrosis including RIP3, TNF and the deubiquitinating enzyme cylindromatosis (CYLD). The authors also reasoned that the intestinal flora might have contributed to the observed phenotype, and

Correspondence: Guido Kroemer
Tel: +33 1 4211 6066; Fax: +33 1 4211 6047
E-mail: kroemer@orange.fr
Abbreviations: CYLD, cylindromatosis; IEC, intestinal epithelial cell; LPS, lipopolysaccharide; RIP, receptor-interacting protein kinase; TLR, Toll-like receptor; TNF, tumor necrosis factor α; TNFR, TNF receptor 1

hence decided to test their observations in the presence of wide-spectrum antibiotics, in germ-free animals and in mice lacking MYD88, a critical mediator of the innate immune response against bacterial products such as lipopolysaccharide (LPS) [9].

FADD^{IEC-KO}/*Ripk3*$^{-/-}$ mice developed normally and did not exhibit any signs of colitis, suggesting that RIP3 is essential for the death of *Fadd*$^{-/-}$ IECs and the associated morbidity. Similar results were obtained when FADD^{IEC-KO} mice were crossed with mice whose IECs express a catalytically inactive variant of CYLD (CYLDΔ932^{IEC} mice), with *Myd88*$^{-/-}$ animals, or when FADD^{IEC-KO} mice were grown in germ-free conditions [9]. These observations suggest that the colic phenotype of FADD^{IEC-KO} mice results from a MYD88-mediated signaling pathway that is ignited by intestinal bacteria and executed by RIP3. In line with this hypothesis, wide-spectrum antibiotics largely ameliorated, though not entirely resolved, colitis in FADD^{IEC-KO} mice, and when germ-free FADD^{IEC-KO} mice were exposed to conventional environmental conditions, they rapidly developed intestinal inflammation [9]. Notably, FADD^{IEC-KO}/*Tnf*$^{-/-}$ mice were partially protected against the colic phenotype, suggesting that intestinal bacteria might activate RIP3 via TNFR, by eliciting Toll-like receptor (TLR) signaling and MYD88-dependent synthesis of TNF (and other cytokines) in the context of an autocrine/paracrine loop (Figure 1A). These molecular cascades appear to be specific for the model of necrotic colitis elicited by the absence of FADD, as the CYLDΔ932^{IEC} genetic background was unable to rescue the colic phenotype of NEMO^{IEC-KO} mice [9], which reportedly depends on the activation of the apoptotic machinery [11].

In addition to colitis (inflammation of the colon), FADD^{IEC-KO} mice also developed enteritis (inflammation of the small intestine), featuring massive necrotic death of IECs and granulocytic infiltration [9]. However, whereas *Ripk3*$^{-/-}$ mice were protected from both enteritis and colitis as induced by the IEC-specific knockout of *Fadd*, enteritis was virtually unaffected by the absence of functional CYLD, MYD88 and TNF, or germ-free conditions [9], implying that another, perhaps cell type-specific, mechanism must exist that accounts for RIP3 activation in the small intestine of FADD^{IEC-KO} mice (Figure 1B). Of note, these animals exhibited a consistent

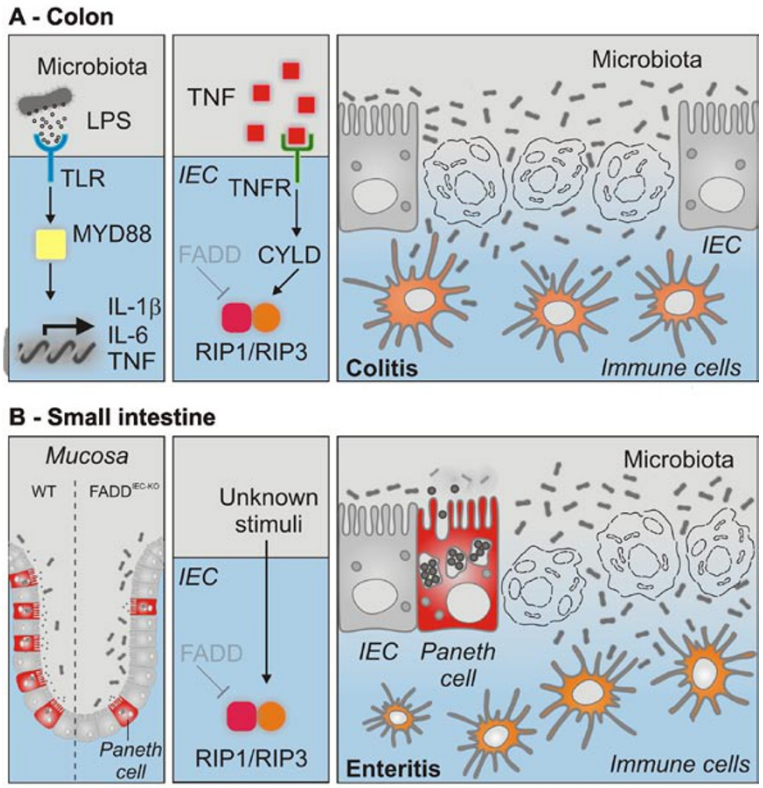

Figure 1 Mechanisms of regulated necrosis in the intestine of FADD^{IEC-KO} mice. **(A)** In the colon of mice bearing an intestinal epithelial cell (IEC)-specific deletion of *Fadd* (FADD^{IEC-KO} mice), the bacterial flora elicits Toll-like receptor (TLR) signaling, resulting in the MYD88-dependent synthesis of pro-inflammatory cytokines including tumor necrosis factor α (TNF), interleukin-1β (IL-1β) and IL-6. These stimulate immune cell infiltration of the intestinal mucosa. Moreover, by binding to TNF receptor 1 (TNFR) on the surface of IECs, TNF activates a signaling pathway that involves the deubiquitinating enzyme cylindromatosis (CYLD) and receptor-interacting protein kinases 1 and 3 (RIP1 and RIP3), resulting in the necrotic death of IECs. Altogether, these molecular and cellular cascades underlie the severe colic disease that characterizes FADD^{IEC-KO} mice. **(B)** FADD^{IEC-KO} mice also manifest the RIP3-dependent loss of IECs in the small intestine and develop enteritis. However, this does not appear to be triggered by the bacterial flora, nor to rely on MYD88-dependent gene transactivation. Interestingly, the small intestine of FADD^{IEC-KO} mice is characterized by a consistent reduction in the Paneth cell compartment. This results in impaired secretion of antimicrobials, in turn allowing for increased bacterial proliferation and (at least theoretically) facilitating the development of the concomitant colic disease. However, the molecular mechanisms that drive the activation of RIP3 in the small intestine of FADD^{IEC-KO} mice remain obscure. In IECs from wild-type (WT) mice, FADD operates as an endogenous inhibitor of RIP3-driven regulated necrosis, thus preventing both colitis and enteritis. LPS, lipopolysaccharide.

reduction in Paneth cells compared to their wild-type littermates [9]. As Paneth cells normally secrete a wide array of antimicrobial peptides into the intestinal lumen, their depletion might facilitate the expansion of the intestinal flora, possibly exacerbating the colic (but not the enteric) phenotype of $FADD^{IEC\text{-}KO}$ mice. Consistent with this notion, Welz *et al.* detected, in the ileum of $FADD^{IEC\text{-}KO}$ mice, impaired expression of antimicrobial factors including lysozyme, α-defensin 20, α-defensin-related sequence 1 and angiogenin 1 [9]. As defects in the autophagic machinery have been associated with notable abnormalities in granule exocytosis by Paneth cells [12, 13], it is tempting to speculate that the signaling pathways leading to RIP3 activation in the small intestine of $FADD^{IEC\text{-}KO}$ mice may be linked to the autophagic machinery. Future investigation is urgently awaited to elucidate these aspects.

Irrespective of these unresolved issues, Welz *et al.* identified FADD as a critical inhibitor of RIP3-dependent regulated necrosis *in vivo*. Together with the fact that the knockout of two essential components of the core machinery for extrinsic apoptosis, namely *Fadd* and *Casp8*, is embryonic lethal unless *Ripk1* and *Ripk3*, respectively, are deleted as well [14, 15], this work suggests the existence of an intimate crosstalk between cell death subroutines that is critical for development as well as for multiple pathophysiological conditions. Nature has invented an elegant design to ensure this crosstalk: while FADD is a positive effector of apoptotic cell death initiated via the extrinsic pathway, it exerts a negative, inhibitory function in regulated necrosis mediated by RIP3.

Acknowledgments

The authors are supported by the Ligue contre le Cancer (équipe labellisée), AXA Chair for Longevity Research, Cancéropôle Ile-de-France, Institut National du Cancer (INCa), Fondation Bettencourt-Schueller, Fondation de France, Fondation pour la Recherche Médicale, Agence National de la Recherche and the European Commission (Apo-Sys, ArtForce, ChemoRes. Death-Train) and the LabEx Immuno-Oncology.

References

1 Kroemer G, Galluzzi L, Vandenabeele P, *et al.* Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ* 2009; **16**:3-11.
2 Casares N, Pequignot MO, Tesniere A, *et al.* Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *J Exp Med* 2005; **202**:1691-1701.
3 Degterev A, Huang Z, Boyce M, *et al.* Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat Chem Biol* 2005; **1**:112-119.
4 Galluzzi L, Vanden Berghe T, Vanlangenakker N, *et al.* Programmed necrosis from molecules to health and disease. *Int Rev Cell Mol Biol* 2011; **289**:1-35.
5 Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G. Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nat Rev Mol Cell Biol* 2010; **11**:700-714.
6 Galluzzi L, Vitale I, Abrams JM, *et al.* Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ* 2011 Jul 15. doi: 10.1038/cdd.2011.96
7 Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell* 2003; **114**:181-190.
8 Yeh WC, Pompa JL, McCurrach ME, *et al.* FADD: essential for embryo development and signaling from some, but not all, inducers of apoptosis. *Science* 1998; **279**:1954-1958.
9 Welz PS, Wullaert A, Vlantis K, *et al.* FADD prevents RIP3-mediated epithelial cell necrosis and chronic intestinal inflammation. *Nature* 2011 Jul 31. doi: 10.1038/nature10273
10 Zhang J, Cado D, Chen A, Kabra NH, Winoto A. Fas-mediated apoptosis and activation-induced T-cell proliferation are defective in mice lacking FADD/Mort1. *Nature* 1998; **392**:296-300.
11 Nenci A, Becker C, Wullaert A, *et al.* Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature* 2007; **446**:557-561.
12 Cadwell K, Liu JY, Brown SL, *et al.* A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 2008; **456**:259-263.
13 Galluzzi L, Kepp O, Zitvogel L, Kroemer G. Bacterial invasion: linking autophagy and innate immunity. *Curr Biol* 2010; **20**:R106-R108.
14 Kaiser WJ, Upton JW, Long AB, *et al.* RIP3 mediates the embryonic lethality of caspase-8-deficient mice. *Nature* 2011; **471**:368-372.
15 Zhang H, Zhou X, McQuade T, *et al.* Functional complementation between FADD and RIP1 in embryos and lymphocytes. *Nature* 2011; **471**:373-376.