Cytokine & Growth Factor Reviews 25 (2014) 157–165

Contents lists available at ScienceDirect

Cytokine & Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Mini review

The *in vivo* significance of necroptosis: Lessons from exploration of caspase-8 function

David Wallach [a,*], Tae-Bong Kang [b], Seung-Hoon Yang [a], Andrew Kovalenko [a]

[a] Department of Biological Chemistry, The Weizmann Institute of Science, 76100 Rehovot, Israel
[b] Department of Biotechnology, College of Biomedical and Health Science, Konkuk University, Chung-Ju 380-701, Republic of Korea

ARTICLE INFO

Article history:
Available online 24 December 2013

Keywords:
Caspase-8
Inflammasome
Inflammation
Necroptosis
Necrosis

ABSTRACT

Emerging evidence indicates that necrotic cell death can be regulated by a specific set of signaling molecules. Studies showing that the same signaling molecules also trigger inflammation, and that when cells die necrotically some of the molecules they release facilitate inflammation, raised the possibility that the death induced by these signaling molecules ("necroptosis") serves *to* trigger inflammation. Here we briefly discuss the work done on the anti-inflammatory function of caspase-8 and its relation to the inhibitory effect of this enzyme on the induction of necroptosis. The studies imply that caspase-8 and the other proximal signaling proteins known to participate in the induction and regulation of necroptosis are too pleiotropic to serve as reliable molecular probes for determining the relative contribution of this death mode to *in vivo* processes.

1. Introduction: a brief historical perspective

Cell death in tissues damaged as a result of inflammation was noticed almost as early as the detection of the leukocyte mobilization events that underlie inflammation. However, definition of the death process was initially so vague that today we cannot tell exactly what was observed in those early studies. Furthermore, we are not even sure what the terms for death used in those studies actually referred to (see [1] for a discussion of such terms). The clear definition of apoptosis in 1972 [2] opened the way to exploration of the relevance of this form of death to inflammation. Some evidence for apoptotic cell death was reported well before that time, but the concept had not been clearly defined [3]. The post-1972 studies disclosed that apoptosis serves an important role in the eventual elimination of leukocytes that mediate various manifestations of inflammation and, as a consequence, in the resolution of this process (*e.g.* [4]). Identification of molecular changes specific to apoptotic cell death, and of signaling mechanisms that induce it, contributed to more accurate recognition of the occurrence of apoptosis in inflammatory processes and provided evidence for its induction in these processes by specific ligands.

In contrast, studies of the occurrence of non-apoptotic cell death in inflammation yielded perplexing data on its role in the process. Accumulating evidence suggested that other cellular changes occurring in inflammation, particularly vascular blockage and activation of granulocytes, precede such death and are the cause of it [5,6]. Yet there was also evidence suggesting that non-apoptotic death plays causal role in inflammation. Of particular note were the studies of Valy Menkin, who suggested that many of the manifestations of inflammation are mediated by molecules generated by injured cells [5], and much later, the work of Polly Matzinger attributing crucial roles to "danger signals" that emanate from injured tissues both in adaptive and in innate immunity [7]. Accumulating knowledge about cellular components that indeed act as "danger signals" (Danger-Associated Molecular Patterns – DAMPs) supports Menkin's and Matzinger's suggestions, though understanding of the exact functional role of individual DAMPs in inflammation is still limited [8]. More recently it was shown that necrotic cell death can be induced by binding of specific agonists such as the cytokine TNF or bacterial endotoxin (LPS) to specific receptors and by subsequent activation of a distinct group of signaling molecules (reviewed in [9]). These findings provided further support for the idea that necrosis might serve to promote inflammation and possibly some other physiological roles as well.

2. Roles of caspase-8 in induction of apoptotic cell death and in necroptosis

Caspase-8 was initially discovered as the proximal enzyme in the induction of apoptotic cell death by receptors of the TNF family [10,11]. It mediates this effect by cleaving and hence

* Corresponding author. Tel.: +972 8 9343941; fax: +972 8 9343165.
E-mail address: d.wallach@weizmann.ac.il (D. Wallach).

1359-6101/$ – see front matter © 2014 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.cytogfr.2013.12.001

activating executioner caspases such as capase-3 and also *via* proteolytic processing of the Bcl-2 family member BID which, once cleaved, initiates pro-apoptotic changes in the mitochondria [12].

Receptors of the TNF family that induce apoptotic cell death can also, in some cells and certain situations, induce necrotic cell death. Two protein kinases, RIPK1 and RIPK3, were recently found to initiate the signaling for necrotic cell death by these and other inducers [13–17]. While serving a crucial function in triggering apoptotic cell-death induction, caspase-8 also plays a major role in restricting the RIPK1/RIPK3-mediated induction of necrotic cell death. It is believed to serve this latter role by cleaving the activated RIPK1 and RIPK3 molecules and also by cleaving the deubiquitinase CYLD, a tumor suppressor that regulates the initiation of necroptosis [18,19].

3. Caspase-8 deficiency *in vivo* stimulates inflammation

Consistent with the crucial role of caspase-8 in the induction of apoptotic cell death through the extrinsic cell-death pathway, knockout of this enzyme in mice was found to block activation of this pathway [20]. However, the main phenotypic changes so far observed in mice as a result of such knockout reflect other functional roles of this enzyme, distinct from the induction of apoptotic death. One of the most obviously apparent of these changes is an increased propensity for inflammation or even its spontaneous emergence. We first observed this functional consequence when studying the phenotype of mice whose hepatocytes were deficient in caspase-8. Under normal conditions this deficiency seemed to have no effect on the mice. However, partial hepatectomy in these mice triggered chronic liver inflammation characterized by increased accumulation of leukocytes, phosphorylation of STAT-3, and hypertrophy of the liver (Fig. 1, [21]). A much more severe inflammatory process was found to develop spontaneously in the mouse skin when *caspase-8* was deleted in the basal cells of the epidermis (Fig. 2, [22]). This acute inflammation first became evident about 3 days after birth and rapidly strengthened, with death occurring a few days later. Deletion of *caspase-8* in enterocytes was similarly shown to trigger chronic inflammation of the intestine [23].

Ubiquitous deletion of *caspase-8* is fatal *in utero* [20]. Therefore, to obtain a broad view of the function of this enzyme we had to find a way to impose partial blockage of its activity throughout the body. Mice expressing subnormal amounts of caspase-8 owing to deletion of one of its two alleles did not display any evident abnormality. However, mice that also expressed an enzymatically inactive allele of *caspase-8* developed chronic inflammation in the skin, as well as in a variety of internal organs, apparently as a result of interference of the mutant enzyme with the function of the enzyme expressed by the wild-type allele (Fig. 3, [22]). This finding further demonstrated that caspase-8 acts to restrict inflammation in various tissues.

4. Several different functions of caspase-8 may contribute to restriction of inflammation

The suspected contribution of necroptosis to inflammation is currently the focus of much research interest. It is therefore important to stress that although caspase-8 deficiency can result in enhanced necroptosis in cultured cells treated with certain inflammation-related ligands, and that if this process occurs *in vivo* it may indeed promote inflammation, the inhibition of necroptosis induction is by no means the only mechanism by which caspase-8 may restrict the initiation of inflammation. Therefore, ablation of this particular effect is not the only factor that can account for the inflammation observed in mice when caspase-8 function is obliterated (Fig. 4). For one thing, apoptotic cells block activation of the innate immune response in other cells [24,25]. Therefore the function that occasioned the discovery of caspase-8, namely its initiation of the extrinsic apoptotic cell death pathway, can contribute to restriction of inflammation through the effects of cells that have died apoptotically as a result of caspase-8 activation ("1" in Fig. 4). Even when cell death does not occur, activation of executioner caspases by the extrinsic cell-death pathway may suppress signaling for inflammation (such as that mediated by the NF-κB transcription factors) by cleaving proteins

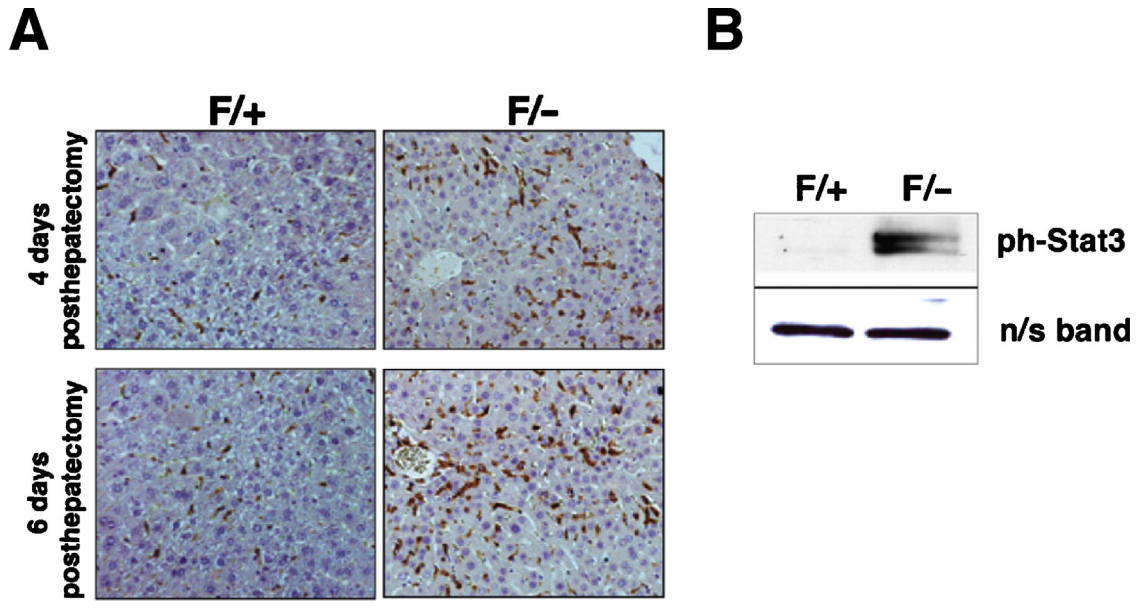

Fig. 1. Caspase-8 deficiency in hepatocytes prompts inflammation the liver after partial hepatectomy. Comparison of the effects of partial hepatectomy on wild-type mice (F/+) and mice with caspase-8-deficient hepatocytes (F/−). (A) Staining with the anti-F4/80 antibody for accumulation of leukocytes in the liver 4 days and 6 days after partial hepatectomy. Magnification: 400×. (B) Western blot analysis of STAT-3 phosphorylation in the liver 14 days after partial hepatectomy.
From [21].