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Molecular determinants of sterile inflammation

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Necrotic cell death alerts the acquired immune system to activate naïve T cells even in the absence of non-self derived molecules (e.g. pathogens). In addition, sterile necrosis leads to innate immune-mediated acute inflammation. The dying cells still represent a threat to the body that should be eliminated by the host immune response. Although the inflammatory response plays important roles in protecting the host and repairing tissues, it can also cause the collateral damage to normal tissues that underlies disease pathogenesis. Tissue resident macrophages recognize the danger signals released from necrotic cells via the pattern recognition receptors and secrete IL-1 that results in acute neutrophilic inflammation. This article will review our current knowledge especially focusing on the role of IL-1 in the sterile necrotic cell death induced inflammation.

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Introduction

The immune system can differentiate among forms of cell death and respond to a perceived threat accordingly. Unlike apoptosis, necrotic cell death induces an acute inflammatory response, even in the absence of pathogens. The innate immune system can sense changes in the integrity of the body. For example, sterile physical damage, such as a burn or bruise, evokes acute inflammation, which consists of the four canonical signs of inflammation: rubor (redness), dolor (pain), calor (heat), and tumor (swelling). These signs were described as early as 2700 years ago. By contrast to the longstanding recognition of the role of inflammation in sterile cell death, our understanding of the mechanism underlying this inflammatory response is just emerging [1–3]. One of the most important steps in this has been the identification of the key mediator interleukin 1 (IL-1) [4••]. Of the 11 members of the IL-1 family, IL-1α and IL-1β has been shown to be responsible for mediating the inflammatory responses to sterile cell death, whereas the IL-1R antagonist (IL-1Ra) is a physiological inhibitor of IL-1. All of IL-1α, IL-1β, and the IL-1Ra bind to IL-1RI signaling receptor which recruits adaptor protein Myd88 and culminated in NF-κB-induced transcription of inflammatory genes. IL-1α is preformed as a active molecule without further modification in cells mainly works as a membrane bound molecule [5]. IL-1β is not expressed under normal homeostatic conditions by bone marrow-derived myeloid cells and is induced as an inactive form by various inflammatory stimuli. Generation and secretion of active IL-1β as well as secretion of IL-1α are mediated by caspase-1 activation on the inflammasome [6••,7].

The mechanism leading to IL-1 production, inflammasome formation, is shared by a variety of stimuli, ranging from sterile stimuli such as dead cells and particulates to microbial stimuli such as bacteria, fungi, or viruses [7]. Although the final 4 canonical signs of inflammation is identical for infectious and sterile causes, the processes of responses to the various stimuli are diverse. Regarding the relationship between cell death and inflammation, apoptosis is a non-inflammatory programmed cell death which is a necessary part of development and tissue homeostasis to remove unwanted cells. Necrosis (oncosis) is an inflammatory non-programmed cell death, which is caused by passive disruption of the plasma membrane that results in releasing of the cytosolic contents to the extracellular space. Other than necrosis, macrophages and dendritic cells undergo an inflammatory programmed cell death, named pyroptosis, that is distinct from apoptosis and necrosis [8]. Pyroptosis is a lytic cell death and mediated by caspase-1 and inflammasome, while apoptosis is non-lytic and mediated by caspase 3/6/7 and apoptosome. Pyroptosis is inflammatory by releasing IL-1 family molecules and other cytosolic contents. More importantly, inflammation caused by necrosis is mediated and/or amplified by macrophages which sometimes undergo pyroptosis. This review focuses on inflammation triggered by sterile dead cells, and specifically on the molecular and cellular mechanisms of both IL-1α and IL-1β.

Danger theory and its extension to acute inflammation

Janeway proposed the principle of discriminating self from non-self by utilizing the recognition of conserved molecular patterns of pathogens, called pathogen-associated molecular patterns or PAMPs (Figure 1) [9]. In this theory, antigen-presenting cells can present the appropriate pathogen-derived peptide to and activate naïve T cells in the presence of PAMPs. This theory was confirmed by identifying Toll-like receptors (TLRs) as the PAMP receptors. TLR engagement on antigen

presenting cells indirectly enhances activated T cell proliferation, differentiation, and survival by promoting the up-regulation of costimulatory molecules and the secretion of proinflammatory cytokines. Nevertheless, this 'Stranger theory' cannot explain some of the well-known immune responses, including the sterile organ transplantation response. Matzinger proposed a theoretical answer to this question, named the 'Danger model' [10]. She explained how the immune system can recognize damage to self by sensing a 'Danger signal'. Danger signals are also capable of activating dendritic cells, which results in the activation of naïve T cells in the same manner as with PAMPs. This theory was confirmed by several studies in which dead cells promoted CD4+ and CD8+ T cell responses [11,12]. The original notion of danger theory was expanded to acute inflammation. TLRs and Nod-like receptors were characterized as pattern recognition receptors, which sense PAMPs and induce acute inflammatory responses [13]. When cells die *in vivo*, neutrophils and monocytes infiltrate the site [14]. This phenomena is also observed in the absence of pathogens; that is, under sterile conditions [15]. The precise mechanism that senses own cell death and induces inflammation was not clear. Advances in the last decade have revealed the molecular identity of the danger signal and the receptors and amplifiers that play a role in this sterile cell death-induced inflammation.

Players in the recognition and amplification of cell-death-induced inflammation

Danger signals

A variety of danger signals have been identified and are classified into two categories on the basis of their actions: (1) molecules that are usually sequestered inside cells and released on necrotic cell death and (2) extracellular matrix, which exposes hidden molecular patterns when fragmented [16]. The hidden intracellular danger signals include uric acid, HMGB1, the myosin heavy chain, SAP130, S100 proteins, ATP, and nucleic acids including mitochondrial DNA [17] (Table 1). Cytokines such as IL-1α and IL-33 have also been identified as danger signals and are released passively from necrotic cells [18–20]. Fragments generated from hyaluronic acid, collagen, elastin, and laminin all stimulate inflammation [16].

Uric acid was the first endogenous molecule identified as functioning as a danger signal via the activation of dendritic cells and enhances the CD8+ T cell responses *in vivo* [21•]. Later uric acid is shown to mediate acute neutrophilic inflammation to necrotic cells in the acute inflammatory responses to the injection of necrotic thymocyte cells or in acetaminophen-induced liver toxicity [22]. Uric acid also stimulates sterile-injury-derived inflammation in the lung [23] or kidney [24]. Uric acid is the end product of the cellular catabolism of purines, and present at near-saturating levels in body fluids and at much higher concentrations in the cytoplasm of cells. In addition, uric acid is produced after cell death by xanthine oxidase [21•,22]. It is speculated that production of uric acid from necrotic cells leads to the phase transition from soluble uric acid to urate crystals (i.e. monosodium urate crystal) around the dying cells with the high sodium extracellular milieu, although the formation of the crystals has not been proven *in vivo*. Monosodium urate (MSU) crystal is formed in human joints which causes acute gouty arthritis [25]. MSU crystals also have been shown to both activate dendritic cells and augment immune responses [21•]. MSU crystals, but not uric acid, induces chemokines and cytokines when added to eosinophils [26]. Collectively, MSU crystals rather than uric acid are the danger signals that can mount both of innate and adaptive immune responses in response to cell death.

Another prototype inflammatory danger signal is HMGB1 protein [27•]. In the normal setting, HMGB1 functions as a chromatin-binding nuclear factor, but it can also be actively secreted by activated immune cells and initiate inflammatory responses [28,29]. HMGB1 is passively released from necrotic cells; by contrast, apoptotic cells modify their chromatin so that HMGB1 binds tightly and thus is not released [27•]. Initially HMGB1 was reported to exert a cytokine like activity including release of TNF-α, IL-1α and IL-β, IL-1Ra, IL-6, IL-8, and MIP-1α and MIP-β when added to mononuclear cells [30]. Later it was shown that recombinant HMGB1 itself induce little or no cytokine secretion [31–33]. At this point, it is shown that HMGB1 forms complex with other molecules including ssDNA, LPS, IL-1β and nucleosomes and exerts inflammatory properties [29]. HMGB1 contributes to evoking inflammation in response to cell death in the sterile liver toxicity model [27•].

Mitochondria is a rich source of danger signals, including mitochondrial DNA, formyl peptides, cytochrome C, and ATP. The highly concentrated danger signals make mitochondria potent stimulators of inflammation [34].

IL-1α also functions as a primary danger signal in certain settings, including the death of dendritic cells or vascular smooth muscle cell [18,35,36]. We confirmed that the necrotic dendritic cells from IL-1α-deficient mice induced reduced inflammation when injected into the peritoneum, compared with cells from wild-type mice [37]. However, necrotic tissue from the liver, brain, and heart of IL-1α-deficient mice had a similar neutrophilic response [37]. Zheng *et al.* also showed the necrosis-induced IL-1α inflammatory activity is highly cell type dependent, and further revealed the molecular mechanism which regulates the activity of IL-1α when released from necrotic tissue [38•]. A decoy IL-1R type II (IL-1R2) which constitutively associates with cytosolic IL-1α and prevents its interaction with IL-1R1 upon release from necrotic cells. Moreover the active caspase-1 cleaves IL-1R2 to make IL-1α accessible to IL-1R1 in