

# Cutting into innate immunity

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**Human caspase-12, unlike that found in the mouse, is not involved in apoptosis and thus its physiological function is unclear. However, a unique polymorphism of human caspase-12 has demonstrated its importance in innate immunity.**

Checks and balances are an essential part of biology. Two chief examples in the immune system are the negative regulation of CD28 costimulation by signaling through cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and inhibition of the cytokine receptor signaling molecule Jak-STAT by suppressor of cytokine signaling (SOCS). Hyperactive T cells in CTLA-4-deficient mice and unregulated cytokine production in SOCS1-deficient mice both lead to lethality<sup>1,2</sup>. Negative regulation of Toll-like receptor (TLR) signaling by the kinase IRAK-M during activation of innate immune cells is another example<sup>3</sup>. However, most recent research in innate immunity has focused on how the TLRs and nucleotide-binding oligomerization domain (Nod) intracellular proteins recognize pathogen-associated molecular patterns and activate macrophages and dendritic cells<sup>4,5</sup>. In *Nature*, Saleh *et al.* now provide new tantalizing evidence for an unexpected new mechanism of broad inhibition of cellular responses to TLR signaling<sup>6</sup>.

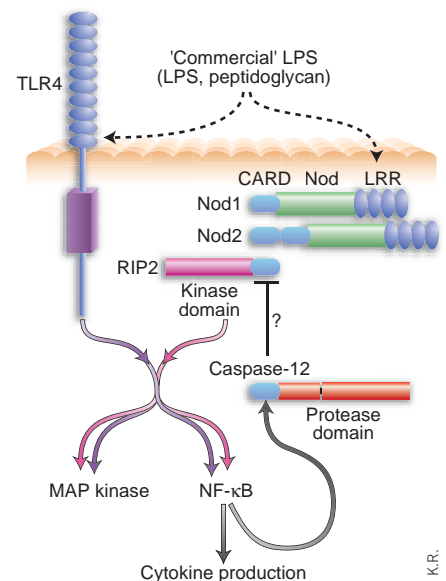
The finding by Saleh *et al.* is unexpected because it involves a member of the caspase family. Caspases are cysteine proteases that are well known for their involvement in apoptosis<sup>7</sup>. Caspase-8 and caspase-9, for example, mediate death receptors and mitochondria pathways of apoptosis, respectively. Caspases 3, 6 and 7 are downstream effector caspases that cleave cellular substrates during cell death. In contrast, a subset of caspases, including caspase-1 and caspase-5, is not involved in apoptosis but is essential for cytokine maturation. These caspases are part of the so-called 'inflammasome' complex, which is crucial for processing of the interleukin 1 (IL-1) precursor into the active form<sup>8</sup>. Caspase-12 is structurally closely related to these 'inflammation' caspases and consists of a protein-protein-interacting caspase recruitment domain (CARD) and a protease domain. The gene encoding caspase-12 is also located in the same chromosomal region as those encoding caspases 1, 4 and 5. Yet

initial studies in mice have assigned an apoptotic function to caspase-12. Mice with caspase-12 deficiency were reported to show less sensitivity to drugs that induce endoplasmic reticulum stress and apoptosis. Furthermore, cytotoxicity mediated by  $\beta$ -amyloid, a protein suggested to be involved in the development of Alzheimer disease, was greatly reduced in caspase-12-deficient neuronal cells<sup>9</sup>.

In contrast to mice, however, most humans carry a gene encoding caspase-12 that contains a premature stop codon between the caspase-12 CARD and protease domain. This difference excludes the possibility of direct involvement of human caspase-12 in initiating apoptosis because the truncated protein (casp12-S) lacks a protease domain. Saleh *et al.* showed that a subset of African-Americans and South Africans carries a polymorphic nucleotide that changes the stop codon to arginine (TGA to CGA). This rare polymorphism allows read-through and subsequent generation of a full-length caspase-12 (casp12-L). However, this caspase-12 cannot form an active protease because unlike the mouse version, human caspase-12 does not have an SHG (serine histidine glycine) box, an element essential for the enzymatic activity of caspases. Thus, human casp12-L is also unlikely to have any direct involvement in apoptosis. Consistent with this hypothesis, Saleh *et al.* demonstrated that apoptosis mediated by endoplasmic reticulum stress is similar in human cells carrying casp12-S or casp12-L<sup>6</sup>.

What is unexpected is the response of peripheral blood cells expressing casp12-L to lipopolysaccharide (LPS) is unexpected. LPS is a component of Gram-negative bacteria outer membranes; commercial preparations of LPS also contain other contaminants, including peptidoglycan found in the cell walls of both Gram-negative and Gram-positive bacteria. LPS stimulates TLR4, whereas peptidoglycan and its derivatives can stimulate the intracellular proteins Nod1 or Nod2 (refs. 4,5). Stimulation of either TLR or Nod proteins leads to a signal cascade that activates MAP kinases and the NF- $\kappa$ B transcription factor, which in turn 'translates' into production of inflammatory cytokines. Saleh *et al.* found that after LPS challenge, casp12-L expression is induced and the stimulated cells

secrete less cytokine than do cells expressing only casp12-S. The effect is global and results in, among other things, decreased production of IL-1, tumor necrosis factor (TNF), IL-5 and interferon- $\gamma$ <sup>6</sup>. Transient transfection of casp12-S or casp12-L into cells resulted in a reduction in NF- $\kappa$ B activation in response to LPS, although the long form of caspase-12 had greater inhibitory activity than the short form. These data suggest that either form of caspase-12 might act as a dominant negative protein to inhibit the TLR or Nod signaling



**Figure 1** Innate immune response signaling and a hypothetical mechanism of feedback inhibition by caspase-12. Innate immunity defends against pathogen infection by detecting pathogen-associated molecular patterns through the leucine-rich repeats (LRRs) of TLRs and intracellular Nod proteins. Commercial preparations of LPS, a component of Gram-negative bacteria, mimic bacterial infection by activating TLR4 and contain peptidoglycan, a component of the bacterial cell wall, which activates Nod1 and/or Nod2. TLR4 and Nod activation initiates a signaling cascade, leading to activation of MAP kinases and NF- $\kappa$ B. Cytokines are subsequently produced to provide immediate protection to pathogens and to influence the development of adaptive immunity. Caspase-12L is upregulated after LPS stimulation and may inhibit cytokine production by dominantly inhibiting the CARD-CARD interaction of Nod proteins with RIP2 through its CARD.

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pathway, but that negative regulation by caspase-12 is more pronounced.

How caspase-12 could inhibit NF- $\kappa$ B is still a subject of speculation, but the explanation could lie in the CARD. Generally, a CARD-containing protein interacts with another CARD protein. Ligand binding to Nod1 or Nod2 leads to oligomerization and recruitment of receptor-interacting protein 2 (RIP2) through their respective CARDS; RIP2 kinase activity can then activate the NF- $\kappa$ B pathway. RIP2-deficient cells are defective in LPS-induced MAP kinase and NF- $\kappa$ B activities<sup>5</sup>. Caspase-12 might prevent the Nod-RIP2 interaction during innate immune responses by binding to the CARDS of Nod1, Nod2 or RIP2 and preventing their association. As caspase-12 is upregulated by LPS signaling, this would then form a negative feedback inhibition mechanism to control innate immune responses (Fig. 1). This hypothesis predicts direct interaction between caspase-12 and RIP2, Nod1 or Nod2 and that elimination of caspase-12 expression by RNA interference should result in increased LPS-induced cytokine production.

But what about mouse caspase-12? There are no published reports of the phenotype of caspase-12-deficient mouse cells after challenge with LPS. Although mouse caspase-12

can be processed into an active protease form, the processing should release a protein fragment containing only N-terminal CARD<sup>9</sup>. This resulting fragment might function similarly to its human counterpart. If this is the case, caspase-12-deficient mice should show enhanced cytokine production when challenged with LPS.

What is the physiological relevance of decreased cytokine production during innate immune responses? The caspase-12 polymorphism correlates positively with increased susceptibility to sepsis and subsequent mortality<sup>6</sup>. This may be counterintuitive at first glance, as TNF-deficient mice are resistant to LPS-induced shock<sup>10</sup> and TNF can cause septic shock when administered alone. Studies in mice have also shown that TNF-neutralizing antibodies could prevent sepsis induced by bacteria infection. However, attempts to prevent sepsis using soluble TNF receptor proteins in human have been mostly unsuccessful<sup>11,12</sup>, suggesting that regulation of cytokine production and sepsis in humans is more complicated than originally envisioned. Cytokines produced during innate immune responses are also essential for controlling pathogen infection. TNF-deficient mice are resistant to LPS shock but succumb easily to bacterial

infection<sup>10</sup>. Perhaps less initial cytokine production in those humans with the caspase-12L polymorphism results in a failure to control the initial bacterial expansion, leading to increased bacterial spread and eventual septic shock due to increased total cytokines in later responses to the infection. Although much work remains to fully elucidate the mechanism of caspase-12 regulation of cytokine responses, targeting this pathway and other regulatory mechanisms of innate immunity may provide a new therapeutic approach for the treatment of sepsis and infectious diseases.

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## Dendritic cells: the immune information management experts

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**Dendritic cells sense and respond to multiple signals that are then conveyed to lymphocytes to direct appropriate immune responses. This flow of information is bidirectional, as antigen-experienced T cells also influence dendritic cell function.**

Dendritic cells (DCs) carry multiple types of information from the site of an infection to the lymph nodes for priming of naive T cells. This information includes antigen specificities as well as cues about the nature of the attacking pathogen and the extent of host damage. In this issue of *Nature Immunology*,

Alpan and colleagues<sup>1</sup> provide evidence for further mechanisms of information management whereby versatile DCs integrate effector function information from previously activated CD4<sup>+</sup> T cells in addition to the other cues provided after infection to selectively 'instruct' the differentiation of naive CD4<sup>+</sup> T cells specific for the same pathogen (Fig. 1).

When exposed to an infection or other insult in peripheral tissues, DCs take up antigens, including apoptotic cells, by macropinocytosis and phagocytosis. Antigens are also acquired by direct infection of DCs or by binding to immunoglobulin Fc, carbohydrate or complement receptors (Fig. 1, left). The DCs then migrate to lymph nodes with their 'cargo' of

antigen while maturing into highly specialized antigen-presenting cells (APCs) that have reduced ability to acquire new antigens<sup>2</sup>. On arrival in the lymph nodes, the DC thus present a set of antigenic epitopes that faithfully reflects the antigenic situation at the site of the infection.

DCs receive additional information (Fig. 1, left) directly from pathogens via 'pattern-recognition receptors', such as the Toll-like receptors (TLRs) that recognize conserved microbial structures, including unmethylated CpG sequences in bacterial DNA, endotoxins such as lipopolysaccharide and double-stranded RNA<sup>3</sup>. These signals induce alterations in the DC so that they subsequently

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