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NLRP1 Joins the Dark Side?

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NLRP1 has long remained an elusive member of the NOD-like-receptor family of innate immune sensors. In this issue of *Immunity*, Masters et al. (2012) describe its role in immune responses to stress and infection.

The human body is under constant attack by various pathogenic members of the microbial world. In order to cope with these threats, an elaborate system of innate receptors evolved to guard the sanctity of tissues, lumen, fluids, and cells. The NOD-like-receptor (NLR) family of innate receptors is specialized in recognizing intracellular threats, given that they continuously scan the cellular cytoplasm for signals of potential hazards (Strowig et al., 2012). The founding members of the NLR family, NOD1 and NOD2, were among the first intracellular innate immune receptors described and are genuine pattern-recognition receptors that recognize and respond to bacterial peptidoglycan fragments by inducing NF-κB-mediated immune responses. In addition to NOD1 and NOD2, several more NLRs have been discovered over the last decade. One of the breakthroughs that led to their identification was the finding that a group of related autoinflammatory disorders, now collectively called cryopyrin-associated periodic syndrome (CAPS), is caused by point mutations in the gene encoding NLRP3 (also called cryopyrin) (Hoffman et al., 2001). In healthy cells, NLRP3 remains in an inactive state. After receptor activation, a multiprotein complex consisting of the adaptor protein ASC and caspase-1 is rapidly formed, enabling the proteolytic activation of caspase-1. Active caspase-1 subsequently processes the leaderless proinflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18), leading to their secretion from the cell. Active caspase-1 also initiates a specific type of inflammatory cell death called pyroptosis (Strowig et al., 2012). Key in regulating this process is the requirement for a priming signal, for instance, LPS (signal 1); only then can NLRP3 activation (signal 2) lead to successful cytokine secretion and pyroptosis. In many CAPS-affected individuals, NLRP3 is believed to be in an "always-on" or hypersensitive state, which leads to uncontrolled and/or continuous inflammation as a result of the loss of requirement for signal 2. In addition to NLRP3, several other NLRs have now been shown to be able to form large protein complexes, or "inflammasomes," that enable the activation of caspase-1. These include NLRC4 (sensing bacterial flagellin and a component of the type III secretion system), AIM2 (sensing DNA), NLRP6 (involved in sensing intestinal barrier disruption), and NLRP1 (Strowig et al., 2012; Elinav et al., 2011).

Although NLRP1 was the first NLR that was shown to form an inflammasome (Martinon et al., 2002), its exact function has remained relatively unclear. In humans, NLRP1 polymorphisms have been associated with a variety of autoimmune diseases, including Addison's disease and vitiligo (Jin et al., 2007). Human NLRP1 has been proposed to recognize muramyl dipeptide, the bacterial peptidoglycan fragment that also activates NOD2 (Faustin et al., 2007). In mice, NLRP1 consists of three homologs, termed NLRP1a, NLRP1b, and NLRP1c (predicted to be a pseudogene). NLRP1b exhibits great variability among different inbred mice strains; the form that is believed to be the "functional" receptor is activated by anthrax lethal toxin (Boyden and Dietrich, 2006). For NLRP1a, no ligand or function has been found as of yet.

In the search for new candidate genes that induce neutrophilia, Masters et al. (2012) performed N-ethyl-N-nitrosourea random mutagenesis in mice and thus produced a mutant presenting with a systemic inflammatory phenotype characterized by massive multiorgan neutrophil influx. The authors identified the responsible mutation as a glutamine-to-proline substitution in the linker region between the nucleotide-binding domain and leucine-rich-repeat domain of NLRP1a (NLRP1a^{Q593P}). Because similar mutations in NLRP3 from CAPS-affected individuals have been described to result in an "always-on" receptor status leading to uncontrolled inflammation, the response of NLRP1a^{Q593P} bone-marrow-derived macrophages to LPS was investigated. Whereas wild-type macrophages required an additional inflammasome stimulus like ATP or alum (signal 2), cells carrying the NLRP1a^{Q593P} mutation responded by producing IL-1β without the need for the crucial second signal; this latter finding is indicative of continuous receptor and inflammasome activation.

To examine the mechanism of NLRP1a^{Q593P}-induced pathology in more detail, Masters et al. (2012) crossed NLRP1a^{Q593P} mice with mice lacking key molecules in inflammasome activation and signaling. Using this approach, the authors identified caspase-1 and interleukin-1R, but not interleukin-1α, as critical for the induction of systemic inflammation, leaving IL-1β as the major cytokine-driving pathology. Interestingly, the adaptor protein ASC was found to be dispensable for this phenotype. ASC is the crucial protein bridge between pyrin domains of NLRs and CARD domains of caspase-1; the lack of a pyrin domain in NLRP1a (which "directly" contains a CARD domain) might explain the ASC independency. This places NLRP1a apart from both NLRP3 (which contains a pyrin domain and requires ASC) and NLRC4 (which contains only a CARD domain but requires ASC for efficient IL-1β secretion) (Broz et al., 2010). Caspase-11, which has been linked to NLRP1 in earlier studies, was also dispensable for NLRP1a^{Q593P}-induced systemic inflammation.

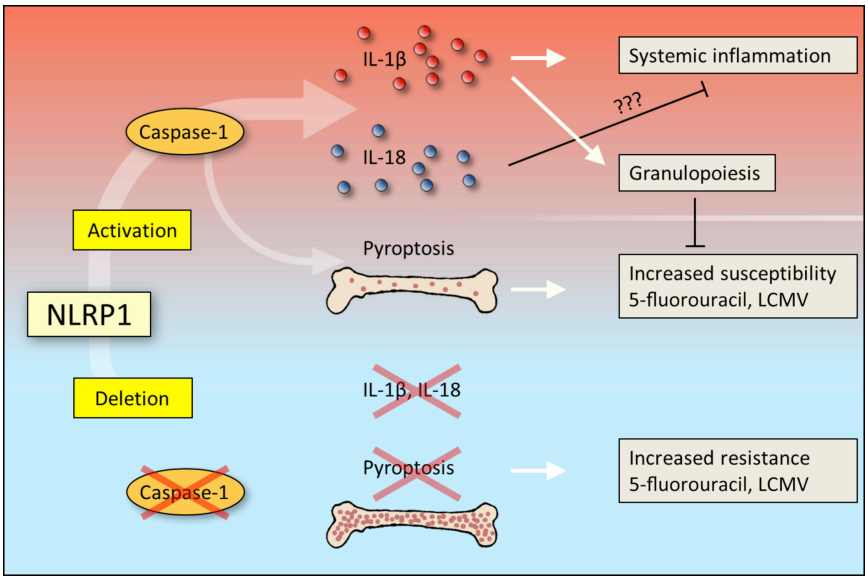

Figure 1. Schematic View of NLRP1-Mediated Immune Responses
Constitutively active NLRP1 results in caspase-1-dependent IL-1β secretion that drives systemic inflammation and in IL-18 that negatively regulates systemic inflammation through an unknown mechanism. NLRP1 and caspase-1-dependent pyroptosis in bone-marrow cells increase the susceptibility to the chemotherapy agent 5-fluorouracil and LCMV infection, which themselves induce massive bone-marrow cell stress and death. IL-1β counteracts the pyroptosis-mediated bone-marrow cell death and susceptibility to 5-flourouracil and LCMV infection through emergency granulopoiesis. Deletion of NLRP1 increases the resistance to 5-fluorouracil and LCMV as a result of the absence of NLRP1-dependent pyroptosis in bone-marrow cells.

IL-18, the second major cytokine produced during NLRP1a^{Q593P} inflammasome activation, had a more complex role in the inflammation phenotype. Through bone-marrow-transplant experiments, Masters et al. (2012) show that large quantities of IL-18 were produced by the hematopoietic cells of NLRP1a^{Q593P} mice. However, deletion of the cytokine in the NLRP1a^{Q593P} background led to increased mortality, most likely as a result of more severe myocarditis. This suggests that IL-18 might play a protective role during systemic inflammation. In an attempt to unravel the mechanism behind this finding, the authors crossed NLRP1a^{Q593P} mice with mice deficient in interferon-γ (IFN-γ), a major cytokine downstream of IL-18. However, deletion of IFN-γ did not phenocopy the IL-18 mutation. Although the precise role of IL-18 was not further elucidated, its induction was shown to be dependent on the presence of the microbiota given that germ-free NLRP1a^{Q593P} mice no longer succumbed more rapidly in the absence of IL-18.

Although the IL-1β-signaling pathway was clearly the driving force behind the inflammatory phenotype in NLRP1a^{Q593P} mice, two additional pathways contributed in a more subtle way that is worth mentioning. First, *Ifng*$^{-/-}$ mice carrying the NLRP1a^{Q593P} mutation developed encephalitis that was not seen in NLRP1a^{Q593P} mice, suggesting a tissue-specific suppressive role for IFN-γ in NLRP1a^{Q593P}-induced inflammation in the brain. Second, NLRP1a^{Q593P} on a T-cell-deficient background resulted in an increase in dermatitis, which apparently is prevented by T cells during systemic IL-1β-driven inflammation.

Unrestricted IL-1β signaling drives powerful systemic inflammation. Using this knowledge, Masters et al. (2012) were able to unmask in NLRP1a^{Q593P} mice an additional phenotype involving NLRP1a^{Q593P}-driven pyroptosis by examining NLRP1a^{Q593P}, *Il1r1*$^{-/-}$ double mutant mice. Characterization of these mice revealed an overall reduced number of hematopoietic stem cells and progenitor cells of both myeloid and lymphoid lineages under homeostatic conditions. Because these deficiencies were not seen in the NLRP1a^{Q593P} mice, the authors concluded that granulopoiesis induced by IL-1β masks the loss of hematopoietic cells that died by uncontrolled pyroptosis. To extend this phenotype to more physiological situations, NLRP1a^{Q593P}, *Il1r1*$^{-/-}$ mice were exposed to 5-fluorouracil chemotherapy or lymphocytic-choriomeningitis-virus (LCMV) infection, which can both induce hematopoietic cell death. In both cases, NLRP1a^{Q593P}, *Il1r1*$^{-/-}$ mice were more susceptible than control *Il1r1*$^{-/-}$ mice. Finally, and in accordance with the model of NLRP1a-driven bone-marrow cell pyroptosis, Masters et al. (2012) showed that mice triple deficient in NLPR1a, NLRP1b, and NLRP1c exhibited increased numbers of hematopoietic stem and progenitor cells and increased resistance to 5-flourouracil treatment and LCMV infection.

The activating mutation in NLRP1a identified by Masters et al. (2012) shows a dramatic inflammatory phenotype (Figure 1). However, when NLRP3 mutations found in CAPS-affected individuals were introduced into mice, an even more striking pathology was observed: most mice died within two weeks (Brydges et al., 2009). This could be a reflection of either the expression profile of NLRP1a versus NLRP3 or the differences in "strength" between the individual mutations. An alternative explanation could be that the different signaling domains in NLRP1a and NLRP3 (CARD versus pyrin) might regulate the level of caspase-1 activation. Intriguingly, human NLRP1 contains both a CARD and a pyrin domain. It would be of great interest to determine the relative contribution of the individual domains to inflammation in both healthy and pathological conditions.

Masters et al. (2012) convincingly show that hyperactive NLRP1a causes massive systemic inflammation and that the absence of NLRP1 in mice leads to a hematopoietic compartment that is better able to deal with local stress and infection. But is it only bad news with NLRP1? Are these negative effects the price that the immune system pays in order to fight off still-unidentified threats? Or does NLRP1-mediated cell death also have beneficial effects? One intriguing hypothesis might be that NLRP1 orchestrates the removal of damaged cells that are no longer able to effectively function or that might cause problems in the future—e.g. mutagenized cells that might become transformed— but at the cost of

decreased ability to survive acute hematopoietic stress. Perhaps the evolutionary pressure on murine NLRP1, shown both by its apparent divergence into three homologs and the existence of multiple forms of NLRP1b, is an example of the intricate balance between the beneficial and harmful functions of NLRP1. The final answer might come with the elucidation of the exact ligands or cellular conditions that activate NLRP1 in mice and humans. This, together with the work by Masters et al. (2012), should begin to clarify the various roles of NLRP1 in host defense and homeostasis.

REFERENCES

Boyden, E.D., and Dietrich, W.F. (2006). Nat. Genet. *38*, 240–244.

Broz, P., von Moltke, J., Jones, J.W., Vance, R.E., and Monack, D.M. (2010). Cell Host Microbe *8*, 471–483.

Brydges, S.D., Mueller, J.L., McGeough, M.D., Pena, C.A., Misaghi, A., Gandhi, C., Putnam, C.D., Boyle, D.L., Firestein, G.S., Horner, A.A., et al. (2009). Immunity *30*, 875–887.

Elinav, E., Strowig, T., Kau, A.L., Henao-Mejia, J., Thaiss, C.A., Booth, C.J., Peaper, D.R., Bertin, J., Eisenbarth, S.C., Gordon, J.I., and Flavell, R.A. (2011). Cell *145*, 745–757.

Faustin, B., Lartigue, L., Bruey, J.M., Luciano, F., Sergienko, E., Bailly-Maitre, B., Volkmann, N., Hanein, D., Rouiller, I., and Reed, J.C. (2007). Mol. Cell *25*, 713–724.

Hoffman, H.M., Mueller, J.L., Broide, D.H., Wanderer, A.A., and Kolodner, R.D. (2001). Nat. Genet. *29*, 301–305.

Jin, Y., Mailloux, C.M., Gowan, K., Riccardi, S.L., LaBerge, G., Bennett, D.C., Fain, P.R., and Spritz, R.A. (2007). N. Engl. J. Med. *356*, 1216–1225.

Martinon, F., Burns, K., and Tschopp, J. (2002). Mol. Cell *10*, 417–426.

Masters, S.L., Gerlic, M., Metcalf, D., Preston, S., Pellegrini, M., O'Donnell, J.A., McArthur, K., Baldwin, T.M., Chevrier, S., Nowell, C.J., et al. (2012). Immunity *37*, this issue, 1009–1023.

Strowig, T., Henao-Mejia, J., Elinav, E., and Flavell, R. (2012). Nature *481*, 278–286.

IL-22 from T Cells: Better Late than Never

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Interleukin-22 (IL-22) enhances mucosal barrier function and is important in antimicrobial host defense. In this issue of *Immunity*, Basu et al. (2012) reveal that Th22 cells are the critical adaptive source of IL-22 during late-phase infection by *Citrobacter rodentium*.

Interleukin-22 (IL-22) belongs to the IL-10 cytokine family and is expressed by innate and adaptive lymphocytes (Ouyang et al., 2011). IL-22 binds to a heterodimeric receptor consisting of IL-22R1 and IL-10R2. IL-22R1, the ligand binding subunit, is expressed by nonhematopoietic cells such as the epithelial cells of the gastrointestinal tract and skin. Therefore, IL-22 functions as a signaling mediator that can connect lymphocytes and epithelial cells. IL-22-IL-22R signaling in epithelial cells results in expression of genes involved in antimicrobial host defense including S100 proteins, defensins, Lipocalin 2, and RegIII-family proteins. IL-22 also induces inflammatory molecules such as chemokines and cytokines including IL-6. In addition, IL-22 has an important function in tissue repair via induction of epithelial cell proliferation and survival. By inducing such genes and by enhancing epithelial barrier function, IL-22 plays an important role in promoting resistance to extracellular pathogens, particularly to Gram-negative pathogens. Indeed, IL-22 signaling is essential for protective immunity to extracellular *Klebsiella pneumoniae* in the lung and *Citrobacter rodentium* in the intestine (Aujla et al., 2008; Sonnenberg et al., 2011; Zheng et al., 2008). IL-22 signaling is also required to prevent systemic dissemination of the *Alcaligenes* bacteria species, which are normally commensal and constitutively reside within the Peyer's patches (Sonnenberg et al., 2012). In addition, IL-22 prevents tissue destruction in several mouse models. In a ConcanavalinA-induced hepatitis model, IL-22 protects from liver injury by enhancing the growth and survival of hepatocytes. In the intestine, IL-22 prevents tissue destruction in dextran sodium sulfate-mediated colitis and in a mouse model of graft versus host disease. In contrast, dysregulated IL-22-IL-22R signaling can promote pathological inflammatory responses in the skin and intestine in mouse models, and the concentration of IL-22 is increased in a variety of human diseases including psoriasis, rheumatoid arthritis, and inflammatory bowel diseases. Furthermore, excessive and aberrant IL-22 signaling results in colon cancer development, as exemplified by mice lacking IL-22-binding protein (IL-22BP), a soluble high-affinity decoy receptor for IL-22 (Huber et al., 2012).

Innate lymphoid cells (ILCs) are one of the major sources of IL-22 (Sonnenberg and Artis, 2012). IL-22-producing ILCs include $CD4^+$ lymphoid-tissue inducer-like cells and $NKp46^+$ natural killer-like cells and are now referred to as group 3 ILCs (ILC3s) (Sonnenberg and Artis, 2012). $IL\text{-}22^+$ ILC3s mainly reside in