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Review

NLRP6 in infection and inflammation

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Abstract

NLRs play fundamental roles in host-defense and inflammatory disorders. NLRP6 is a newly characterized member of this family that inhibits NF-κB and MAP-kinase dependent immune signaling to hamper anti-microbial defense. Further, NLRP6 regulates intestinal inflammation by maintaining gut microbiota composition. In this review, we examine the recent studies and emphasize the key functions regulated by NLRP6.

Keywords: NLRP6; NLR; Innate immunity; Infection; Microbiota; Intestinal homeostasis

1. Introduction

Members of the Nod-Like Receptor (NLR) family play fundamental roles in immunity and host defense. Gain-of-function mutations in NLRs are correlated with several inflammatory disorders. Further, certain NLRs play critical roles in pathogen recognition and activate downstream signaling cascades resulting in anti-microbial defense [1–3]. However, a functional role for most of the family members is yet to be elucidated. While pattern-recognition receptors such as Toll-like receptors (TLRs) are well defined, the biology of NLRs as a family is only beginning to be understood. In contrast to TLRs that mostly sense extracellular microbes, NLRs sense microbial or danger stimuli in the cytoplasm [1,2]. Remarkably, pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs), which are conserved among pathogens and non-pathogens alike. As such, they are unable to differentiate microbes based on their pathogenicity [4,5]. However, invasion of the host–cytoplasm is a more serious threat and NLRs are aptly localized to respond to these insults.

NLRs are comprised of 23 family members in humans and 34 in the mouse genome [6,7]. Structurally, NLRP6 resembles the well-studied member NLRP3 consisting of a N-terminal Pyrin domain, a central NOD domain and C-terminal leucine-rich repeats. Following activation, NLRP3, as also NLRC4 and NLRP1, forms a multimeric structure known as the 'inflammasome' typically leading to oligomerization of the adaptor protein ASC and activation of the cysteine protease procaspase-1 [8], which further cleaves precursors of IL-1β and IL-18 to their biologically active forms [6,9–12]. NLRP3 is activated in response to numerous diverse stimuli by equally distinct mechanisms that include changes in ion flux, generation of reactive oxygen species and lysosomal disruption. In contrast to a prior belief of direct sensing, NLRC4 was recently shown to attain specificity through distinct NAIP proteins that recognize a functional bacterial type III or IV secretion system or flagellin and subsequently activate NLRC4 [13,14]. While it is beginning to be deciphered for a limited set of NLRs, the specific ligand and activation mechanisms for most of the family members remains unknown.

Characterization of the NLR family members is essential to gain deeper understanding of the functions of these receptors in immunity and inflammatory disorders. Recently, NLRC5 was described to act as a transactivator of MHC class I genes (CITA) [15]. NLRP10 was proposed to initiate adaptive immunity by dendritic cells and its ablation resulted in increased

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susceptibility to *Candida albicans* infection [16,17]. NLRP7 was demonstrated to assemble an inflammasome in response to microbial diacylated lipopeptides in human macrophages [18]. In the last few years, a number of NLRs were described that function to restrain immune signaling. NLRX1 inhibited inflammatory cytokine production via the MAVS-RIG-I pathway [19]. NLRC3 and NLRP12 inhibited TLR-dependent activation of NF-κB [20–22]. Similarly, our lab described role for NLRP6 in inhibiting TLR-dependent NF-κB and MAP-kinase signaling [23]. Below, we will discuss in detail the newly discovered roles of NLRP6 in inflammation and host-defense.

2. NLRP6 protects against colitis and colitis-associated carcinogenesis

Ulcerative colitis is an inflammatory disorder of the gastrointestinal tract affecting 1.4 million people in the United States [24]. During DSS-induced experimental colitis in mice, tight junctions between epithelial cells in the colon are disrupted allowing permeability of commensal bacteria and bacterial ligands into the underlying lamina propria thereby promoting gratuitous inflammation. In this model, *Nlrp6*-deficient mice displayed enhanced susceptibility accompanied with reduced serum IL-18 levels [25]. Interestingly, this was correlated to altered fecal microbiota composition in *Nlrp6*-deficient mice as demonstrated by increased representation of the bacterial phyla Bacteroidetes (family Prevotellaceae) and TM7. These microbial communities also displayed enhanced expansion in $Asc^{-/-}$ and $Casp1^{-/-}$ mice that demonstrated equal susceptibility to colitis thereby implying a role for NLRP6 in inflammasome formation. Furthermore, the colitogenic gut microbiota could establish increased representation in WT mice when co-housed with any of the above-mentioned knock-out animals [25]. The expansion of Prevotellaceae stimulated elevated levels of CCL5 (RANTES) in colons of $Asc^{-/-}$, $Nlrp6^{-/-}$ and in susceptible co-housed WT mice [25]. However, despite comparable acquisition of Prevotellaceae, $Ccl5^{-/-}$ mice exhibited significant resistance to DSS-induced colitis [25] thus implying a downstream function for CCL5 in enhancing disease (Fig. 1). Finally, in agreement with a role for IL-18, mice deficient in *il18* also displayed expansion of Prevotellaceae and thus enhanced colon CCL5 expression. These findings thus demonstrate a critical role for inflammasome-dependent IL-18 production in maintaining composition of the intestinal microbiota (Fig. 1). However, certain differences exist in the fecal microbiota composition of $Asc^{-/-}$ and $Nlrp6^{-/-}$ mice compared to $il18^{-/-}$ mice [25] suggesting the presence of additional IL-18-independent mechanisms of microbiota regulation. Altered microbiota composition was also demonstrated to result in exacerbated non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in *Nlrp6*- or *il18*-deficient mice [26]. However, whether IL-18 directly regulates the growth of bacteria belonging to family Prevotellaceae needs to be examined.

Chronic inflammation during colitis predisposes individuals to an increased risk of colorectal cancer, the second leading cause of death due to cancer in the US. In 2009, 136,000 people were diagnosed with colorectal cancer resulting in

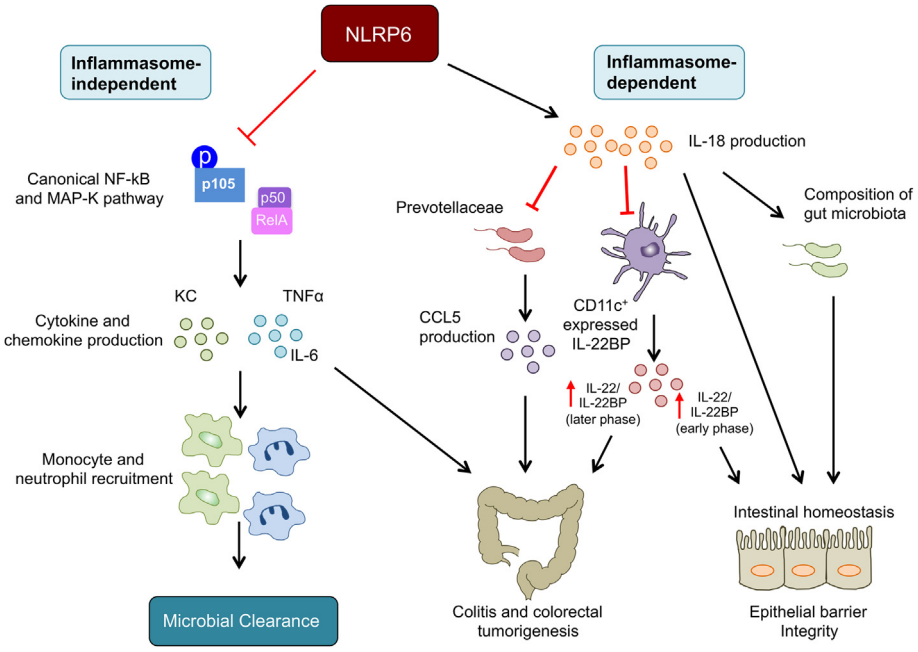

Fig. 1. NLRP6 regulates inflammasome-dependent and inflammasome-independent functions. Left: NLRP6 inhibits canonical NF-κB and MAP-kinase signaling in an inflammasome-independent manner. Thus, deficiency in *Nlrp6* results in enhanced secretion of cytokines and chemokines. Consequently, increased monocytes and neutrophils are recruited thereby augmenting anti-microbial defense. Right: NLRP6 protects against intestinal homeostasis by maintaining gut microbiota composition and IL-18 production in an inflammasome-dependent manner. *Nlrp6* ablation results in an altered microbiota with enhanced expression of Prevotellaceae and enhanced production of CCL5 thus promoting colitis. IL-18 production also regulates epithelial barrier integrity. Further, IL-18 regulates the levels of IL-22BP released by dendritic cells to adjust IL-22/IL-22BP ratio. Increased IL-22/IL-22BP ratio in the early phase of intestinal damage promotes colonic epithelial cell repair while its increase in the later phase promotes colitis-associated carcinogenesis.