Nucleotide-Binding Oligomerization Domain-Like Receptors and Inflammasomes in the Pathogenesis of Non-Microbial Inflammation and Diseases

D. Randal Mason  Paul L. Beck  Daniel A. Muruve

Department of Medicine, Immunology Research Group and the Institute of Infection, Immunity and Inflammation, University of Calgary, Calgary, Alta., Canada

Key Words
Nucleotide-binding oligomerization domain-like receptor  •  Inflammasome  •  Non-microbial inflammation  •  Chronic disease

Abstract
The nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) or nucleotide-binding domain leucine-rich repeat-containing family of genes plays an important role in the development of innate immune responses. Some family members are known to form multiprotein complexes known as inflammasomes that regulate the processing and secretion of proinflammatory mediators, such as interleukin-1β and interleukin-18. Activity of the inflammasome is triggered not only by microbial infection, but also by a wide range of both exogenous and endogenous noninfectious stimuli. Consequently, the dysregulation of inflammasome activity is associated with numerous proinflammatory, non-microbial human diseases. The discovery of NLRP3 gene mutations in autoinflammatory diseases such as Muckle-Wells syndrome has led to the association of NLRs in the pathogenesis of many non-microbial diseases that include arthritis, neurodegenerative disorders, metabolic disorders (obesity and diabetes), cardiovascular disease (atherosclerosis, myocardial infarction), inflammatory bowel disease, kidney disease and hypersensitivity dermatitis. A number of NLRs are also associated with human disease in the absence of inflammasome activity, suggesting additional roles for NLRs in the regulation of inflammation and disease. This review serves to provide a summary of NLR-associated diseases and, where possible, the mechanisms behind the associations.

Introduction
Interest in the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) or nucleotide-binding domain leucine-rich repeat-containing family of genes by both researchers and clinicians is evident by the hundreds of articles published on the topics of NLR structure, function and impact on human pathologies over the past decade. With ongoing discovery and classification, extensive effort has been put forth to understand how this family of proteins modulates inflammatory processes and directs both innate and adaptive responses.

Most pattern recognition receptors including Toll-like receptors (TLRs), RIG-like receptors and C-type lectin receptors are recognized for their ability to initiate immune responses to infections following activation by pathogen-associated molecular patterns (PAMPs). While PAMPs can also activate NLRs, many of these genes act as cytosolic innate sensors to damage/danger-associated molecular patterns (DAMPs) that include a wide range of noninfectious, environmental and host-derived stimuli. As such, the NLRs are capable of initiating and sustaining strong inflammatory responses to tissue damage or other
pathophysiologic perturbations that are central to the pathogenesis of many common non-microbial diseases. This review serves to outline the role of NLRs in autoinflammatory, autoimmune, degenerative and other non-infectious disorders.

The NLR Family of Genes

The NLR family was discovered through genome mining that revealed 22 related human NLR genes [1]. The NLRs are a class of cytosolic innate sensors, with most sharing a conserved tripartite structure consisting of an N-terminal caspase recruitment domain (CARD) or pyrin domain (PYD), followed by a central nucleotide-binding (NACHT) domain and a C-terminal leucine-rich repeat (LRR) domain. The CARD and PYD are known sites of protein-protein interactions, while the LRR domain is thought to play a role in ligand sensing and auto-regulation. The NACHT domain is named after the four proteins for which this domain was initially characterized (NAIP, CIITA, HET-E and TP1) and functions as an NTPase with preferential affinity for ATP and GTP. This NTPase activity is involved in the capacity of NLRs to assume an active state capable of oligomerization [2].

The NLRs are categorized based on conserved N-terminal domains or on phylogenetic similarities (table 1). In the former, genes are group based on the presence of N-terminal domains that include PYD, CARD, baculovirus inhibitory repeat-like (BIR) or acidic transactivating activity and a C-terminal leucine-rich repeat domain (LRR). The NLRs are divided into several subfamilies, including the pyrin, the CARD, the BIR, and the acidic transactivating activity subfamilies.

Table 1. Domain structures and general function of NLR family members

<table>
<thead>
<tr>
<th>Member</th>
<th>Domain structure</th>
<th>Proposed function</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLRA</td>
<td>(CARD)-AD-NACHT-LRR</td>
<td>MHC II gene expression</td>
</tr>
<tr>
<td>NLRB</td>
<td>BIR-NACHT-LRR</td>
<td>antiapoptotic</td>
</tr>
<tr>
<td>NLRB1</td>
<td>NAIP (NLRB1)</td>
<td>antiapoptotic</td>
</tr>
<tr>
<td>NLRD1</td>
<td>CARD-NACHT-LRR</td>
<td>NF-κB pathway, autophagy</td>
</tr>
<tr>
<td>NLRD2</td>
<td>CARD-CARD-NACHT-LRR</td>
<td>NF-κB pathway, autophagy</td>
</tr>
<tr>
<td>NLRD3</td>
<td>NACHT-LRR</td>
<td>unknown</td>
</tr>
<tr>
<td>NLRD4</td>
<td>CARD-CARD-NACHT-LRR</td>
<td>NF-κB pathway, autophagy</td>
</tr>
<tr>
<td>NLRD5</td>
<td>CARD-NACHT-LRR</td>
<td>inflammasome activity</td>
</tr>
<tr>
<td>NLRD6</td>
<td>NACHT-LRR</td>
<td>inflammasome activity, MHC I gene expression</td>
</tr>
<tr>
<td>NLRX1</td>
<td>X-NACHT-LRR</td>
<td>inhibition of antiviral responses</td>
</tr>
<tr>
<td>NLRP1</td>
<td>PYD-NACHT-LRR-FIIND-CARD</td>
<td>inflammasome activity</td>
</tr>
<tr>
<td>NLRP2</td>
<td>PYD-NACHT-LRR</td>
<td>inhibition of NF-κB pathway</td>
</tr>
<tr>
<td>NLRP3</td>
<td>PYD-NACHT-LRR</td>
<td>inflammasome activity, other</td>
</tr>
<tr>
<td>NLRP4</td>
<td>PYD-NACHT-LRR</td>
<td>autophagy, NF-κB pathway</td>
</tr>
<tr>
<td>NLRP5</td>
<td>PYD-NACHT-LRR</td>
<td>reproduction and development</td>
</tr>
<tr>
<td>NLRP6</td>
<td>PYD-NACHT-LRR</td>
<td>inflammasome activity</td>
</tr>
<tr>
<td>NLRP7</td>
<td>PYD-NACHT-LRR</td>
<td>reproduction and development, inflammasome regulation</td>
</tr>
<tr>
<td>NLRP8</td>
<td>PYD-NACHT-LRR</td>
<td>unknown</td>
</tr>
<tr>
<td>NLRP9</td>
<td>PYD-NACHT-LRR</td>
<td>unknown</td>
</tr>
<tr>
<td>NLRP10</td>
<td>PYD-NACHT-LRR</td>
<td>inflammasome regulation</td>
</tr>
<tr>
<td>NLRP11</td>
<td>PYD-NACHT-LRR</td>
<td>unknown</td>
</tr>
<tr>
<td>NLRP12</td>
<td>PYD-NACHT-LRR</td>
<td>inhibition of NF-κB pathway</td>
</tr>
<tr>
<td>NLRP13</td>
<td>PYD-NACHT-LRR</td>
<td>unknown</td>
</tr>
<tr>
<td>NLRP14</td>
<td>PYD-NACHT-LRR</td>
<td>reproduction and development</td>
</tr>
</tbody>
</table>

Inflammasome components

| ASC       | PYD-CARD              | inflammasome activity                                  |
| Caspase-1 | CARD-p20/p10          | inflammasome activity                                  |

CIITA = Class II, major histocompatibility complex, transactivator; CARD = caspase recruitment domain; AD = acidic activation domain; MHC = major histocompatibility complex; NF-κB = nuclear factor-κB; FIIND = domain with function to find; X = X domain; P20/P10 = caspase-1 catalytic subunits.
tion domains producing PYD-containing NLR (NLRP), NLRC, NLRB and NLRA subfamilies [1]. The second classification divides genes based on phylogenetic similarities to produce NOD, interleukin (IL)-1β-converting enzyme protease-activating factor (IPAF) and NLRP subfamilies [2]. The NOD subfamily consists of membrane-associated NLRs that are predominantly characterized by their involvement in autophagy and induction of nuclear factor-κB. There are many extensive reviews focused on the NOD subfamily, and thus, this article will not focus on the biology of these NLRs.

There are 14 identified human NLRPs, all of which contain PYD, NACHT and LRR domains with the exception of NLRP10, which lacks the LRR. In addition to the three conserved domains, NLRP1 contains FIIND (domain with function to find) and CARD domains which contribute to its function in inflammasome formation (see below) [3]. While some members of this subfamily are well understood, there are a number of NLRPs for which their function has yet to be fully determined (table 1) [2].

The IPAF subfamily consists of two members, IPAF (or NLRC4, i.e. CARD containing NLR4) and neuronal apoptosis inhibitory protein (NAIP, or NLRB1, i.e. BIR containing NLR1). NLRC4 is noted for its ability to form an inflammasome that responds primarily to bacteria with type III/IV secretion systems and PAMPs such as flagellin and type III secretion apparatus [2]. In contrast, NAIP is known for its antiapoptotic properties [4].

Both the NLRP and IPAF members have gained notoriety in recent years due to the ability of some members to form multiprotein inflammasome complexes capable of responding to both pathogen- and host-derived signals. These inflammasomes are primarily caspase-1-activating platforms that induce the maturation of pro-cytokines, most notably IL-1β and IL-18 (fig. 1). The most widely

**Fig. 1.** a Overview of inflammasome regulation and activation. b Inflammasome-independent NLR functions. RLR = RIG-like receptor; MHC = major histocompatibility complex.
studied is the NLRP3 inflammasome, which consists of NLRP3, caspase-1 and the adaptor protein apoptosis-associated speck-like protein (ASC). Upon activation, NLRP3, ASC and caspase-1 oligomerize via homotypic interactions between respective PYD and CARD protein domains. The resulting high-molecular-weight complex forms a ring-like structure (the inflammasome) that triggers caspase-1 autoprocessing into an active cysteine protease [2]. Among other functions, active caspase-1 cleaves members of the IL-1 superfamily from their inactive precursor forms to active secreted cytokines (fig. 1). The NLRP1 and NLRC4 inflammasomes contain structural differences to the NRLP3 inflammasome, but serve the common purpose of caspase-1 activation.

The increasing interest in the role of NLRs in the pathogenesis of non-microbial diseases stems from several observations. First, activating NLRP3 mutations are known to cause a variety of autoinflammatory disease in humans that comprise the cryopyrin-associated periodic syndromes (CAPS, see below) [5]. Second, as caspase-1-activating platforms, the range of potential inflammasome effector molecules extends beyond IL-1β and IL-18 implicating NLRs in a variety of cellular functions that can impact disease. Caspase-1 regulates the release of proinflammatory secretary proteins that lack a signal peptide such as high-mobility group box 1 and fibroblast growth factor 2 [6–8]. Caspase-1 also participates in apoptosis and the processing of non-cytokine substrates such as PARP and the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase and aldolase [9, 10]. Thus, the absence of IL-1β or IL-18 does not necessarily exclude a role for inflammasome-forming NLRs in disease pathogenesis.

Third, the mechanisms leading to inflammasome assembly and activation, while not completely understood, provide a basis for NLR activation as a consequence of cellular stress and tissue injury. Several models examining NLRP3 inflammasome activation, for example, have been proposed to include a dependence on reactive oxygen species [11, 12], lysosomal destabilization [13] or the ATP-gated ion channel, P2X7 receptor [14, see ref. 2 for a detailed review of inflammasome structure and regulation]. Finally, while the formation and activity of inflammasomes are critical in a number of pathologies, there are a number of NLR-associated disorders that are independent of inflammasomes, suggesting a variety of functions for the NLRs. In this regard, NLRs (aside from NOD1/ NOD2) have been shown to regulate a wide range of cellular functions including nuclear factor-κB signaling (NLRP2, NLRP4, NLRP12, NLRC5) [15–18], RIG-like re-
that R258W mutation in the hematopoietic cell compartment, particularly antigen-presenting cells, primarily contributed to the disease phenotype that was effectively reversed by blocking IL-17A and the IL-1 receptor [32]. Similarly, CAPS-inducing Nlrp3 mutants L351P and A350V drove both innate (inflammasome/IL-1β) and T-cell-dependent inflammation [31]. L351P and A350V Nlrp3 transgenic mice crossed onto a Rag1−/− background (unable to generate mature T or B cells) had improved survival, but the mice were only fully protected on an ASC−/− background, suggesting primarily innate (i.e. inflammasome-dependent) inflammation in this model [31]. These in vivo models have shed significant light onto the molecular events in the pathogenesis of CAPS and have opened the door to potential therapeutics.

**Joint Disorders**

The most common disorders associated with joint inflammation and pain is gout, osteoarthritis and rheumatoid arthritis (RA). Gout is characterized by pain and inflammation of the peripheral joints due to the deposition of monosodium urate (MSU) crystals. MSU has been characterized as a danger signal that is released from dying cells. In 2006, Martinon et al. [33] demonstrated that MSU and calcium pyrophosphate crystals were able to directly activate the NLRP3 inflammasome in human monocytes and murine macrophages assessed through caspase-1 and IL-1β maturation. The effect of MSU-induced IL-1β activation was abrogated in mice or cells deficient in Nlrp3, ASC and caspase-1. Transgenic mice lacking the LRR domain of Nlrp3 display reduced inflammatory responses to MSU similar to Nlrp3−/− mice, indicating that the LRR domain is important in MSU sensing [34]. The efficacy of inflammasome inhibition in the amelioration of MSU-induced inflammation led to a pilot study showing the efficacy of anakinra (recombinant IL-1 receptor antagonist, IL-1Ra) in reducing inflammation and clinical symptoms in patients with gout [35].

RA is a chronic inflammatory disorder involving inflammation of the synovium, leading to degradation of the articular cartilage and stiffening of the joints. While RA most commonly affects the synovium, it is considered a systemic disorder that can manifest in other areas of the body such as the lungs and vasculature. Polymorphisms in NLRP3 link significantly to RA [36], and NLRP1 and NLRP3 gene expression is elevated in peripheral blood mononuclear cells of RA patients [37]. Consistent with these observations, increased expression of NLRP3 and ASC is seen in the synovium of RA patients, including the endothelium and infiltrating leukocytes [38]. Expression of numerous NLRPs such as NLRP1, NLRP3, NLRP6, NLRP10, NLRP12, NLRP14 and other inflammasome components including ASC, pro-IL-1β and caspase-1 has also been detected in fibroblast-like synoviocytes [39]. Interestingly, NLRP expression was also detected in the synovium of osteoarthritis patients, in a manner that was generally indistinguishable from RA [39]. Anakinra is effective in the treatment of RA, suggesting that inflammasome activation and secretion of IL-1β are key processes involved in RA and associated disease pathogenesis [40].

**Brain, CNS and Neuronal Disorders**

Work published on inflammation in the CNS and specific CNS-related disorders has primarily investigated the roles of IL-1β, IL-18 and caspase-1. Human and animal models of diseases such as Huntington's and Parkinson's disease as well as amyotrophic lateral sclerosis have
all demonstrated involvement of these proinflammatory mediators, but have yet to demonstrate a direct association with any particular NLR protein [41–43]. However, NLRs may play a role in degenerative neurological disorders. NLRP1 and NLRP5 have been directly implicated in neuronal death following serum starvation in vitro and cerebral artery occlusion in vivo [44]. Similarly, NLRs have been shown to contribute to Alzheimer’s disease (AD), a form of dementia characterized by progressive loss of neurons and synapses coinciding with the accumulation of amyloid-β plaques and neurofibrillary tangles, which are aggregates of hyperphosphorylated τ protein. Fibrillar amyloid-β induces phagosomal damage in microglia resulting in proinflammatory chemokine and cytokine secretion, including IL-1β release [45]. Nlrp3–/– and ASC–/– microglia demonstrated a significant reduction in the proinflammatory mediators when treated with amyloid-β, suggesting that the NLRP3 inflammasome plays an important role in this context [45]. A screen of AD patients also revealed elevated levels of caspase-1, which may be associated with NLR inflammasome activity [46]. Taken together, these results suggest a role for NLR proteins in the pathogenesis of AD and possibly other neurodegenerative diseases.

Multiple sclerosis (MS) is an autoimmune disorder characterized by chronic inflammation and demyelination of the neurons of the CNS. Increased levels of caspase-1 have been observed in MS patients, in cell culture studies and in the mouse model of MS, i.e. experimental autoimmune encephalomyelitis (EAE) [47, 48]. In MS patients, increased levels of caspase-1 and IL-18, but not IL-1β, were detected in peripheral blood mononcytes [48]. Consistent with these findings, ASC and Nlrp3 were found to play a role in the progression of disease in the EAE model [49, 50]. The study by Gris and colleagues [49] demonstrates the requirement for NLRP3 in the expression of interferon (IFN)-γ and IL-17, which drive Th1 and Th17 immune responses and the neurological injury in this model. Nlrp3–/– mice demonstrated reduced inflammation, injury and clinical manifestations of the disease corresponding with a reduction in IFN-γ and IL-17-secreting CD4+ T cells. IL-18 was the critical effector downstream of NLRP3, since IL-18 and Nlrp3-deficient mice exhibited similar phenotypes [49]. In contrast, another group reported a predominant role for ASC and caspase-1 but not for NLRP3 in EAE progression [50]. ASC–/– but not Nlrp3–/– mice displayed a reduction in clinical score as well as in IFN-γ and IL-17-secreting CD4+ T cells obtained from the draining lymph nodes and the spinal cord. Together, the existing data strongly suggest a role for the inflammasome in the pathogenesis of MS; however, the discrepancy observed in recent studies regarding the pertinent genes may relate to the sensitivity of the EAE model to environmental conditions.

Cardiovascular Diseases

Research over the past decade indicates that inflammation is a key mediator in the development of cardiovascular diseases. Recent genome-wide association studies found that polymorphisms in the NLRP3 locus were concordant with fibrinogen gene variants that are associated with increased circulating fibrinogen levels, a risk factor for cardiovascular disease [56]. Consistent with these observations, inflammasome involvement has been demonstrated in the context of cardiac ischemia-reperfusion (I/R) injury [57]. Kawaguchi et al. [57] demonstrated increased ASC expression in the hearts of patients following myocardial infarction and less cardiac injury following I/R injury in ASC–/– and caspase-1–/– mice. Compared to WT mice, ASC–/– and caspase-1–/– mice had better left ventricular function and less inflammation following ischemia. Interestingly, inflammasome activation occurred not only in infiltrating leukocytes but also in cardiac fibroblasts, likely triggered by reactive oxygen species and K+ efflux. ASC has also been shown to play a role in a vascular injury/restenosis model in mice [58]. Increased ASC expression in macrophages and vascular smooth muscle cells were observed at the site of wire-in-
duced vascular injury and coincided with neointimal formation [58]. Neointimal formation and restenosis was reduced in ASC−/− mice and corresponded, not surprisingly, with less IL-1β and IL-18 expression. Unlike the findings in the myocardium, ASC-dependent restenosis following vascular injury was entirely dependent on infiltrating leukocytes.

The deposition of cholesterol on arterial walls and subsequent uptake by circulating monocytes precedes differentiation of monocytes into foam cells. Foam cells are key mediators in the development of atherosclerotic lesions and it is believed that inflammation is driven by the conversion of cholesterol into a crystalline form within these macrophages [59]. Studies have shown that cholesterol crystals activate the NLRP3 inflammasome in macrophages [60]. In the absence of NLRP3, ASC or caspase-1, IL-1β maturation is abolished in response to cholesterol crystals, an effect that was also lost in cathepsin-B- and cathepsin-L-deficient cells providing evidence for the lysosomal disruption/cathepsin activation model of the inflammasome. In vivo, low-density lipoprotein receptor-deficient mice that received Nlrp3−/− or ASC−/− bone marrow experienced a reduced burden of atherosclerotic lesions, providing strong support for a role of the inflammasome in the pathogenesis of vascular disease. In an interesting twist, Freigang et al. [61] outlined the role of NF-E2-related 2 (Nrf2), an oxidative stress responsive transcription factor in the cholesterol crystal model. Apolipoprotein E (ApoE)−/− mice have been shown to have an increased susceptibility for the development of atherosclerosis. Using an ApoE−/− background, Nrf2−/− ApoE−/− mice were protected from IL-1-dependent atherosclerotic damage while Nrf2+/− ApoE−/− mice were not [61]. Cholesterol crystal-induced IL-1β production was not only significantly reduced in caspase-1−/− and Nlrp3−/− macrophages, but also in Nrf2−/− cells, indicating a dependency on both the NLRP3 inflammasome and Nrf2, reinforcing the link between inflammasome activation and the upregulation of antioxidant genes [62, 63].

Finally, NLRs and the inflammasome are also indirectly linked to cardiovascular disease, given their roles in obesity and insulin resistance that are important cardiovascular risk factors (see below for further details). Taken together, these studies show a significant role for the NLRP3 inflammasome in cardiovascular disease and in disease states associated with increased cardiovascular risk.

**Pulmonary Diseases**

NLRs are known to be involved in the host response and control of pulmonary infections that involve diverse pathogens such as viruses, bacteria and mycobacteria [64]. The role of NLRs in airway diseases associated with autoimmunity and environmental antigens is less well defined than in microbial infections, but still substantial. Screening a cohort of patients with systemic sclerosis-related fibrosing alveolitis demonstrated the presence of NLRP1 single nucleotide polymorphisms (SNPs) as a significant risk factor within patient populations measured against healthy controls, suggesting that NLRs play a role in non-microbial driven inflammation and lung disease [65].

Chronic obstructive pulmonary disease is commonly broken down into two categories: chronic bronchitis and emphysema. Chronic bronchitis is characterized by inflammation and damage to the upper airways, while emphysema is characterized by inflammation and damage to the alveoli. The inflammasome has been show to play a role in mouse models of lung injury and emphysema. Nasal installation of elastase in mice results in the degradation of extracellular matrix and the alveolar wall to produce an emphysema-like state. Increased production of proinflammatory cytokines, such as tumor necrosis factor-α, IL-6 and IL-1β, and increased neutrophil infiltration are associated with elastase administration in WT mice [66]. IL-1Ra administered to mice prior to elastase exhibited less inflammation and tissue damage. In addition, when elastase was administered to ASC−/−, IL-1R−/− and MyD88−/− mice, the markers of inflammation and injury were significantly less, indicating significant involvement of the inflammasome and the IL-1 signaling axis in the pathogenesis of disease. Furthermore, the efficacy of uricase in reducing elastase-mediated injury suggested that the underlying mechanism may involve uric acid-induced activation of the NLRP3 inflammasome [66]. Similarly, using bleomycin to induce acute injury, chronic inflammation and lung fibrosis, the IL-1 signaling axis was shown to contribute significantly to this disease model [67]. IL-1R−/− or MyD88−/− mice were resistant to bleomycin-induced injury, and pretreatment with IL-1Ra also protected the lungs from extensive inflammation and fibrosis. Bleomycin-induced inflammation and tissue damage was also substantially decreased in ASC−/− mice, and a subsequent study confirmed the involvement of NLRP3 and caspase-1 [67, 68]. It was hypothesized that the underlying pathogenesis of bleomycin-induced injury involved uric acid release and inflammasome activation. Indeed, exogenous uric acid induced lung inflammation and reducing uric acid with the xanthine oxidase inhibitor allopurinol or uricase decreased bleomycin-induced inflammation and injury [68].
The role of NLRP3 and the inflammasome in asthma is controversial. Kool et al. [69] demonstrated that release of uric acid upon allergen exposure leads to the development of a Th2-mediated response that is independent of the NLRP3 inflammasome. Using ovalbumin (OVA) + alum and house dust mite-allergic lung inflammation models, an increase in uric acid was observed that coincided with eosinophil and lymphocyte accumulation in the lung and the development of a Th2 immune response. The response was attenuated when allergens were coadministered with uricase, suggesting that uric acid was essential to the response. Increased uric acid was also seen in bronchoalveolar lavage fluid of asthma patients. Despite the clear adjuvant effect provided by uric acid in these models, administration of OVA + alum or house dust mite to Nlrp3–/–, ASC–/– or IL-1R–/– mice did not reduce eosinophil or lymphocyte recruitment, suggesting that the NLRP3 inflammasome or IL-1 signaling was not essential to the response. Rather, phosphoinositide-OH-3 kinase and Syk were needed for directing the development of the Th2 response. Rather, phosphoinositide-OH-3 kinase and Syk were needed for uric acid-mediated adjuvanticity [69]. These findings directly contradict another study using the OVA asthma model demonstrating a substantial reduction in inflammation and leukocyte recruitment to the lung in Nlrp3–/– and IL-1R–/– mice compared to WT mice [70]. Inflammasome and IL-1-deficient mice displayed significantly lower Th2-associated cytokines, IL-13-secreting CD4+ T cells and dendritic cells in the draining lymph nodes following OVA challenge. While controversial at the moment, further research may clarify the roles of NLRs and inflammasomes in the development of Th2 immune responses and allergic lung diseases.

Severe chronic lung diseases can result from the pulmonary exposure to particulates such as silica and asbestos (asbestosis, silicosis). Caspase-1 and IL-1β maturation were activated by silica and asbestos in THP-1 cells and WT murine macrophages in an NLRP3 and ASC-dependent manner [71]. Blocking reactive oxygen species or phagocytosis by treatment with cytochalasin D prior to exposure to particulates prevented inflammasome activity, indicating that endocytosis of the particles was required for activation. These results were recapitulated in vivo by Cassel et al. [72], demonstrating the importance of the NLRP3 inflammasome in response to pulmonary exposure to asbestos and silica. In a similar vein, the NLRP3 inflammasome and IL-1α have been implicated in the pulmonary response to nanoparticles silica dioxide (SiO2) and titanium dioxide (TiO2) that are commonly used in product manufacturing [73]. IL-1β maturation and caspase-1 activation occurred in THP-1 cells and murine macrophages stimulated with TiO2 and SiO2, a response abrogated in Nlrp3 –/– and ASC–/– macrophages. In contrast to the effect of other particulate matter, such as MSU, the activation of the inflammasome by nanoparticles did not require cytochalasin-D-dependent cytoskeletal rearrangement. In vivo, pulmonary exposure to TiO2 resulted in inflammasome activation and neutrophil recruitment in WT mice. Nlrp3–/–, ASC–/– and caspase-1–/– mice demonstrated attenuated inflammatory responses, indicating that the NLRP3 inflammasome was only partially mediating the inflammation following nanoparticle exposure. In this regard, IL-1α and signaling via the IL-1R was crucial [73].

Liver Diseases

An interest in researching the role of NLRs in sterile injury to the liver has emerged due to the realization that NLRs respond strongly to host-derived, endogenous antigens. McDonald et al. [74] used a focal hepatic burn model to assess the impact of the NLRP3 inflammasome on neutrophil recruitment. Nlrp3–/– and ASC–/– mice exhibited decreased levels of IL-1β which was essential to trigger the cascade of leukocyte recruitment to the site of injury. As mentioned in the introduction, evidence suggests that ATP binding to the P2X7 receptor and subsequent K+ efflux stimulates activation of the inflammasome. Consistent with this model, decreasing the level of extracellular ATP using apyrase or pharmacological inhibition of the P2X7 receptor resulted in impaired neutrophil recruitment. Likewise, neutrophil adherence was decreased and IL-1β release was abrogated in P2X7–/– mice [74].

The NLRP3 inflammasome may also play a role in toxin-induced hepatic injury. Acetaminophen (AAP) overdose results in an accumulation of toxic metabolites that deplete the liver of the antioxidant glutathione causing damage and potentially failure of the liver. Nlrp3–/–, ASC–/– and caspase-1–/– mice showed increased survival rates and decreased liver damage in histological analysis using a model of AAP-mediated hepatotoxicity [75]. Survival rates were the same for WT as for IPAF–/– mice, suggesting that the injury is driven specifically through the NLRP3 inflammasome. In addition, TLR9 was needed to induce transcription and production of pro-IL-1β and pro-IL-18, an effect that was reduced by aspirin. Subsequently, TLR9 deficiency or coadministration of aspirin reduced AAP-induced liver injury. These findings were followed up by another group that applied the same methodology but were unable to demonstrate a reduction in AAP-mediated injury in mice lacking the various com-
ponents of the NLRP3 inflammasome and IL-1 signaling [76]. The contradictory findings leave the subject open to debate as to the involvement of the NLRP3 inflammasome in AAP-mediated hepatotoxicity. Finally, inflammasome involvement also contributes to hepatic stellate cell activation and experimental liver fibrosis induced by carbon tetrachloride and thioacetamide. MSU upregulated transforming growth factor-β and collagen-1 expression in isolated WT hepatic stellate cells but not in ASC−/− hepatic stellate cells. Consequently, intraperitoneal administration of carbon tetrachloride increased the expression of transforming growth factor-β and collagen-1 and subsequent liver fibrosis in mice that was reduced in Nlrp3−/− and ASC−/− animals [77]. Similar findings were seen in ASC−/− mice receiving thioacetamide. In summary, substantial evidence exists that the inflammasome plays a role in liver injury.

Metabolic Disorders

Recent studies suggest an important role for mitochondrial stress in driving inflammation and inflammasome activation, which may indicate a role for NLRs in a number of different metabolic disorders [12]. Zhou et al. [11] first demonstrated a role for NLRP3 in glucose metabolism. Thioredoxin-interacting protein (TXNIP), a negative regulator of the antioxidant enzyme thioredoxin, was found to regulate NLRP3 activation during oxidative stress. TXNIP plays a significant role in glucose metabolism, with its expression increased by hyperglycemia and decreased by insulin. In pancreatic islet cells, hyperglycemia triggered a small, albeit significant, amount of IL-1β secretion, a response that was absent in Nlrp3−/− or TXNIP−/− cells. Subsequent in vivo studies confirmed that Nlrp3−/− and TXNIP−/− mice display a similar improvement in glucose tolerance and insulin sensitivity compared to WT mice fed a high fat diet [11]. Masters et al. [78] also examined the role of the Nlrp3 inflammasome in experimental diabetes. Islet amyloid polypeptide (IAPP), a protein implicated in the pathogenesis of type 2 diabetes mellitus, was found to activate the Nlrp3 inflammasome in macrophages via the phagolysosome. IAPP transgenic mice also displayed an increase in IL-1β expression in pancreatic islets, suggesting that IAPP may contribute to diabetes pathogenesis via the inflammasome. In contrast to previous studies, TXNIP was not required for Nlrp3 inflammasome activation in macrophages. While the proposed mechanisms may differ, these studies highlight the potential role for the inflammasome in obesity-related diseases such as diabetes.

More recently, several studies have emerged that confirm a role for the NLRP3 inflammasome in obesity, adipocyte biology and insulin sensitivity. Stienstra et al. [79] demonstrated that caspase-1-dependent production of IL-1β had a significant negative impact on adipogenesis, insulin resistance and obesity in general, strengthening the inflammation-related link to disease pathogenesis. Caspase-1−/− cells or antagonism of IL-1β significantly improved the adipocyte phenotype and insulin sensitivity. In obesity models in vivo, both caspase-1 and IL-1β were found to be increased in adipose tissues, and caspase-1 deficiency or inhibition substantially improved insulin sensitivity, adipose tissue morphology and composition, fat oxidation rates, bone mass and glucose tolerance. Similar observations were also seen in Nlrp3−/− fat cells and mice but not as pronounced as in caspase-1 deficiency [79]. Vandannagars et al. [80] also noted increased NLRP3 and IL-1β expression in adipose tissue that correlated with the degree of obesity and insulin resistance. Ceramide generated from fatty acids was found to be an NLRP3 trigger, and Nlrp3−/− mice had improvements in insulin sensitivity, hepatic steatosis and an alteration in adipose tissue macrophages towards an M2 phenotype. In addition, significant changes were also noted in T-cell populations in various fat compartments with a significant alteration in the numbers or phenotype of CD4+ and CD8+ lymphocytes. NLRP3 inflammasome activity associated with obesity may be driven by the presence of saturated fatty acids such as palmitate [81]. Palmitate, which is increased in high fat diets, activated the NLRP3 inflammasome via reactive oxygen species production, reduced autophagy mediated by downregulation of the antioxidant enzyme AMP-activated protein kinase. Similarly, in MSU-induced gouty arthritis, fatty acids can act on the TLR2 to increase IL-1β in an ASC- and caspase-1-dependent fashion [82]. Csak et al. [83] found that saturated fatty acids induce inflammasome activation which in concert with endotoxin exposure may be the underlying mechanism involved in the development of nonalcoholic steatohepatitis. Together, these studies confirm important roles for the inflammasome in metabolic disorders, including insulin signaling. The inflammasome may represent a key pathway that links diet and obesity to inflammation, type 2 diabetes and organ dysfunction.

Kidney Diseases

Despite a large number of diseases associated with inflammation of the kidney and altered function, there are very few publications on the topic of NLR expression...
and activity in the kidney. Chronic kidney disease (CKD) is a progressive loss of renal function that occurs over an extended period of time. The most common causes of CKD are diabetes mellitus, glomerulonephritis and hypertension. Options for treatment are lacking and the focus lies on slowing the progression of the disease. Several lines of investigation have suggested a role for the inflammasome in CKD. P2X7−/− mice experienced less tubular injury, inflammation and renal fibrosis compared to WT mice with renal injury induced by unilateral ureteric obstruction (UUO) [84]. Similarly, biglycan and hyaluronan, two endogenous danger signals that activate NLRP3, also play a role in renal injury [85, 86]. The direct role of NLRP3 was subsequently established in human CKD and in mice [87]. Expression of NLRP3, IL-1β, IL-18 and caspase-1 activation increased over the time course of UUO in mice confirming inflammasome activity in this model. Compared to WT mice, kidneys from Nlrp3−/− animals displayed less tubular damage, interstitial fibrosis and inflammatory cell infiltrates. Interestingly, studies using bone marrow chimeric mice revealed a function for NLRP3 in both the renal and hematopoietic compartments. In human CKD, NLRP3 gene expression was increased in a variety of non-diabetic kidney diseases and correlated with kidney function [87].

The inflammasome has also been shown to play a role in I/R injury in the kidney. Caspase-1 was shown to play a significant role in I/R injury in the kidney mainly through its effects on IL-18 maturation [88]. Mice deficient in caspase-1 experienced less renal failure, neutrophil recruitment and pathological tubular injury compared to WT mice. Iyer et al. [89] first showed reduced tubular injury following I/R of the kidney in Nlrp3−/−, ASC−/− and caspase-1−/− mice. Inflammasome activation in macrophages was triggered by the release of mitochondrial ATP from necrotic cells acting via P2X7, in addition to priming by endogenous DAMPs, such as hyaluronic acid and biglycan. Shigeoka et al. [90] reported similar findings regarding the role of NLRP3 in renal I/R. Kidney function and tubular injury was preserved in Nlrp3−/− mice compared to WT counterparts. Interestingly and in contrast to prior studies [88, 89], although Nlrp3−/− mice exhibited a reduction in the production of mature IL-1β and IL-18, mice deficient in IL-18, IL-1R, ASC and caspase-1 were not protected from I/R injury and exhibited a similar phenotype as WT controls [90]. These observations, in addition to bone marrow chimeric studies, suggested that I/R injury in the kidney was mediated by inflammasome-independent NLRP3 function in the epithelial cell compartment. While no mechanism was proposed, the results were consistent with a role for epithelial NLRP3 in the UUO model of renal injury [87].

Gastrointestinal Disorders

The gastrointestinal tract is a unique area of the body in terms of immunological responses and tolerance to non-self antigens. The constant presence of dietary antigens and commensal microbiota necessitates the presence of a fortified mucosal barrier for the intestinal epithelial cells. Beneath the single epithelial layer lies a network of leukocytes, including macrophages, T and B cells ready to respond to any disruptions to intestinal homeostasis assessed by the presence of PAMPs and DAMPs. The responses of the NLRP3 and IPAF inflammasomes are well characterized for enteric organisms [64]. Thus, it is not surprising that the NLRs play a major role in inflammatory bowel disease (IBD) that includes Crohn’s disease (CD) and ulcerative colitis. Both are characterized by chronic intestinal inflammation, abdominal pain, rectal bleeding and diarrhea. While CD can affect all regions of the gastrointestinal tract (most commonly involving the terminal ileum and colon), ulcerative colitis is generally restricted to the colon and rectum.

Differential expression of several NLRs was observed in Paneth cells from IBD patients [91]. NLRP1 and NLRP7 gene expression was upregulated, while NLRP8 and NLRP11 expression was significantly downregulated in IBD patients. The roles of NLRP7, NLRP8 and NLRP11 are not well defined in the literature and it will be interesting to see if and how they play a role in the regulation of IBD. In most organ systems, the functioning NLRP3 inflammasome appears to play a role in the etiology and exacerbation of inflammatory disorders. However, SNP array studies have outlined the significance of NLRP3 polymorphisms in CD, indicating a hypofunctional NLRP3 allele associated with increased susceptibility to CD [92]. Mouse models of IBD have generally confirmed a protective role for NLRP3 in the dextran sulfate sodium (DSS) and 2,4,6-trinitrobenzenesulfonic acid-induced models of colitis. Using these models, several studies have demonstrated that Nlrp3−/−, ASC−/− and caspase-1−/− mice were much more susceptible to epithelial barrier damage, suggesting a role for the NLRP3 inflammasome in the maintenance of intestinal homeostasis [93–96]. Inflammasome-deficient mice exhibited increased inflammation, tissue damage and decreased survival. In addition, Hirota et al. [95] observed a significant difference in the microbiota between Nlrp3−/− and WT mice and...
speculate that it may be reflective of the inability of the Nlrp3–/– mouse to properly maintain intestinal homeostasis. Together, these studies support the notion that the NLRP3 inflammasome plays a key role in intestinal inflammation and in damage induced through experimental models of colitis.

In addition to the NLRP3 inflammasome, recent evidence suggests that the NLRP6 inflammasome is also a key regulator of intestinal homeostasis. Similar to the findings with NLRP3, mice deficient in Nlrp6 are more susceptible to DSS-induced colitis that coincides with a significantly altered gut microbiota (dysbiosis) [97]. The co-housing and cross-fostering of ASC–/–, caspase-1–/– and Nlrp6–/– mice with WT mice revealed that the increased susceptibility to DSS-induced injury could be transferred to WT mice and is thought to be driven by dysbiosis [97]. Chimeric studies indicated that the expression of NLRP6 in non-hematopoietic cells, particularly the epithelium, is essential for the maintenance of normal microbiota [97] while expression in hematopoietic cells may function to negatively regulate tumorigenesis [98].

Skin Disorders

Similarly to the gut, the skin serves as a barrier to prevent access of potential pathogens and environmental hazards. The defenses of the skin include commensal bacteria, antimicrobial peptides and a system of leukocytes beneath the skin that serve to protect the host. Research into the role of NLR proteins in protection of the skin and mediation of inflammatory disorders is beginning to emerge. Vitiligo is an autoimmune disorder characterized by a loss of skin pigmentation due to the destruction of melanocytes. Several studies have been published correlating NLRP1 and NLRP3 SNPs with the disorder [99, 100]. Polymorphisms in NLRP1, NLRP3 and NLRP12 have also been associated with atopic dermatitis [101]. Consistent with these data, the inflammasome has been demonstrated in the pathogenesis of contact hypersensitivity (CHS), a T-cell-mediated form of delayed-type hypersensitivity in the skin in response to allergens. CHS is mediated in part by the NLRP3 inflammasome as demonstrated through the use of a chemically induced hypersensitivity model [102, 103]. Primary keratinocytes and keratinocyte-derived cells express many different NLRPs and are capable of forming functional inflammasomes. Stimulation with contact sensitizers such as trinitrochlorobenzene and dinitro-1-fluorobenzene was sufficient to induce inflammasome activation and IL-1β maturation in an ASC- and caspase-dependent manner. Edema and vasodilation characteristics of CHS were attenuated in Nlrp3–/–, ASC–/– and IL-1R–/– mice following topical application of trinitrochlorobenzene or dinitro-1-fluorobenzene [103]. Nlrp3–/– and ASC–/– mice were also resistant to trinitrophenyl chloride-induced CHS, an effect that likely occurs in the early sensitization step [102]. NLRP12 is also associated with CHS. The use of Nlrp12–/– mice did not affect levels of IL-1β in a CHS model, but the lack of NLRP12 appeared to disrupt proper migration of dendritic cells and neutrophils following topical application of oxazolone [104]. The disruption in leukocyte recruitment was not associated with a decreased expression of the CCR7 or CXCR4 chemokines, as Nlrp12–/– mice exhibited levels similar to WT mice. This novel study on the function of NLRP12 reminds us that the NLR proteins likely perform many important, inflammasome-independent functions that are yet to be defined.

Future Perspectives

The identification of NLRs in disease has increased substantially over the past 10 years. However, it is clear that much more work is required to elucidate their exact biology, mechanism of activation and, in the case of inflammasomes, their complete effector functions. While most work with the NLR family of proteins has focused on their role in driving inflammation through inflammasome formation (and IL-1β and IL-18 in particular), there is evidence to suggest that some NLRs (aside from NOD1 and NOD2) function independently of inflammasome formation and may even act in an anti-inflammatory capacity. The disease mechanisms related to inflammasome-independent NLR function remains to be determined. Similarly, even when the multiprotein complex of the inflammasome is formed, evidence of the numerous substrates of caspase-1 suggests that the inflammasome complex may have functions well beyond the activation of members of the IL-1 family. These data reveal that NLRs likely cannot be grouped together based on function, but rather exhibit diverse functions to regulate the consequences of microbial and non-microbial injury.

Finally, while a significant amount of data has now linked the NLRs and the inflammasomes to a variety of common diseases, the translation to therapeutics is required. The identification of the NLRPs and the inflammasomes has resulted in the successful application of anti-IL-1β therapies, such as IL-1Ra, which have proven to


Mason/Beck/Muruve
be effective in reducing symptoms in a number of auto-inflammatory diseases, such as CAPS, gout and RA, and are being explored as a treatment for a wide variety of other disorders [5]. However, the effector functions of the NLRs and the inflammasome are diverse. Thus, specific inhibitors of NLRs and their associated proteins and effectors are needed to realize the full therapeutic benefit of the recent focus on this family of genes over the past decade. Continued interest in researching NLRs will undoubtedly broaden our understanding of their functions and how they may be manipulated in the treatment of a myriad of disorders.

References


5 Neven B, Prieur AM, Quartier dit Maire P: NLRs and Disease J Innate Immun 2012;4:16–30


Acknowledgements

P.L.B and D.A.M. are supported by salary awards and operating grants from Alberta Innovates Health Solutions and the Canadian Institutes for Health Research. D.A.M. holds a Tier 2 Canada Research Chair.

3178–3184.

376.


28 Mason/Beck/Muruve


