



HHS Public Access

Author manuscript

Immunol Res. Author manuscript; available in PMC 2016 November 11.

Published in final edited form as:

Immunol Res. 2014 August ; 59(1-3): 142–152. doi:10.1007/s12026-014-8544-x.

NLR proteins and parasitic disease

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Abstract

Parasitic diseases are a serious global health concern. Many of the most common and most severe parasitic diseases, including Chagas' disease, leishmaniasis, and schistosomiasis, are also classified as neglected tropical diseases and are comparatively less studied than infectious diseases prevalent in high income nations. The NLRs (nucleotide-binding domain leucine-rich-repeat-containing proteins) are cytosolic proteins known to be involved in pathogen detection and host response. The role of NLRs in the host response to parasitic infection is just beginning to be understood. The NLR proteins NOD1 and NOD2 have been shown to contribute to immune responses during *Trypanosoma cruzi* infection, *Toxoplasma gondii* infection, and murine cerebral malaria. The NLRP3 inflammasome is activated by *T. cruzi* and *Leishmania amazonensis* but also induces pathology during infection with schistosomes or malaria. Both the NLRP1 and NLRP3 inflammasomes respond to *T. gondii* infection. The NLRs may play crucial roles in human immune responses during parasitic infection, usually acting as innate immune sensors and driving the inflammatory response against invading parasites. However, this inflammatory response can either kill the invading parasite or be responsible for destructive pathology. Therefore, understanding the role of the NLR proteins will be critical to understanding the host defense against parasites as well as the fine balance between homeostasis and parasitic disease.

Keywords

Parasitology; NLR proteins; Innate immune defense; Inflammasomes

Introduction

The NLR family

The NLR proteins are a family of 22 mammalian genes that have diverse functions in innate immunity and inflammation. The best characterized of these proteins, NLRP1, NLRP3, and NLRC4, act as cytosolic sensors and regulate cytokine secretion and cell death pathways. The family shares common structural motifs: a central nucleotide-binding domain (NACHT), a C-terminal leucine-rich-repeat (LRR) domain that has been implicated in ligand sensing, and an N-terminal effector domain that is either a CARD (caspase-1 activation and recruitment), BIR (baculovirus inhibitor of apoptosis protein repeat) domain or a PYD (pyrin) domain. The NLRP group members contain a PYD; the NAIPs include a BIR domain, and the NLRC group proteins contain a CARD domain (Fig. 1) [1].

At least three of these family members (NLRP1, NLRP3, and NLRC4) and the IFI202X/IFI16 family member AIM2 are known to function in the assembly of a large molecular weight complex known as the inflammasome, where they act as a scaffold along with the adaptor protein ASC (apoptosis-associated speck-like protein containing a CARD) leading to caspase-1 activation, and consequent activation and release of IL-1 β and IL-18 [2, 3]. Other NLRs (NOD1, NOD2) are known to regulate inflammatory immune responses, whereas the molecular functions of many NLR proteins have yet to be discovered. Several NLRs, including some of the non-inflammasome-activating NLRs, play crucial roles in the host adaptive response to pathogens regulating cellular migration, goblet cell-induced mucous secretion, and negative regulation of inflammatory pathways [4–11].

Inflammasome activation

The formation of multiprotein inflammasome complexes constitutes a major pro-inflammatory pathway of the innate immune system. Signals that trigger activation are unique to each of the NLR proteins. Inflammasomes function in tandem with a priming signal, often ligation of a TLR, leading to production of pro-IL-1 β . Upon activation by the appropriate ligand, the NLR couples with ASC and procaspase-1, converting caspase-1 to its active form that then cleaving the precursors of IL-1 β and IL-18 into their mature secreted forms. Release of these cytokines leads to further inflammatory cascades in the host. All of the inflammasomes except the AIM2 inflammasome contain an NLR family protein that acts as pattern recognition receptor. NLRP1, NLRP3, NLRC4, and AIM2 have been confirmed to form inflammasome complexes with caspase-1 (Fig. 2) [1–3]. Other NLR proteins such as NLRP6 and NLRP12 have been proposed to form inflammasomes, although there is not conclusive evidence of their inclusion in an inflammasome complex [4, 12].

The NLRP1 inflammasome activation is activated by anthrax lethal toxin and by muramyl dipeptide (MDP), a bacterial peptidoglycan motif. Although there is no direct evidence of MDP-NLRP1 binding, it is proposed that pathogen-derived muramyl dipeptide binds NLRP1 in the cytosol, altering the NLRP1 conformation and allowing oligomerization and caspase-1 inflammasome assembly. Human NLRP1 has its own CARD domain enabling its direct binding to caspase-1; thus, the presence of the ASC adaptor is not essential [13, 14]. Mice have three paralogs of NLRP1. Among these, NLRP1b is activated by *Bacillus*

anthracis lethal toxin and is required by the host to combat infection, i.e., animals with the lethal toxin-sensitive Nlrp1b allele have increased resistance to *B. anthracis* infection [15].

The NLRC4 inflammasome involves at least two NLR proteins, NLRC4 and a partner NAIP, to detect cytosolic bacterial proteins. In humans, a single NAIP detects the needle structure of the bacterial TSS3 system [16]. In mice, NAIP1 detects the needle, NAIP2 detects the rod of TSS3, and NAIP 5 and 6 detect bacterial flagellin directly [16–21]. The exact mechanism of the interaction between the NAIP and NLRC4 is unknown, although NLRC4 must be phosphorylated for inflammasome activation [22].

The AIM2 inflammasome, while not containing an NLR protein, assembles in a similar manner as the NLRP3 inflammasome. Thus, AIM2 recruits the ASC adaptor and caspase-1, which autolytically activates and cleaves pro-IL-1 β and pro-IL-18 into their mature and secreted forms. The AIM2 HIN-200 domain functions as a sensor for cytosolic double-stranded DNA [23–25]. This allows the AIM2 inflammasome to sense a variety of threats that introduce dsDNA to the cytosol, including bacteria, DNA viruses, parasites, and internal damage [23, 26–28].

The NLRP3 inflammasome is the best-studied of the inflammasomes with the largest and most diverse list of agonists. Activation requires NLRP3 and the ASC adaptor as well as caspase-1. Pathogen-derived products, sterile crystalline molecules, and endogenous stimuli such as ATP are all capable of activating the NLRP3 inflammasome. The precise mechanism of NLRP3 activation by these diverse agonists is uncertain, although many agonists cause cation flux (including K⁺ efflux and increased cytosolic Ca²⁺ concentration) that may cause mitochondrial disruption, which is sensed by NLRP3 [29–31]. NLRP3 inflammasomes are activated by viral, bacterial, fungal, and parasitic pathogens. The precise function of the NLRP3 inflammasome in these infections is varied. Production of IL-1 β and IL-18 can trigger a protective adaptive immune response or a cascade of inflammatory cytokines and chemokines, which can result in pathology [3]. Indeed, pathologic consequences of unregulated inflammasome activation in autoinflammatory disease, such as the cryopyrin-associated periodic syndrome, are specifically treated with the recombinant IL-1 receptor antagonist anakinra. The ability of inflammasomes to induce sterile inflammation followed by a strong adaptive response has been exploited in the use of alum, a potent NLRP3 activator, and the NLRP1 agonist MDP as vaccine adjuvants [32].

Functions of non-inflammasome-forming NLRs

The non-inflammasome-forming NLRs, including NOD1, NOD2, NLRC3, NLRC5, NLRP2, NLRP6, NLRP10, NLRP12, and NLRX1, have diverse functions in immunity.

NOD1 and NOD2 have been well studied. Human mutations in NOD1 are linked to atopic disease and asthma, and in NOD2 are closely linked with the autoinflammatory disorder Crohn's disease and Blau syndrome [33]. Both NOD1 and NOD2 function in sensing bacteria, and it is hypothesized that improper recognition of gut microbiota due to the absence or malfunction of NOD1 and NOD2 leads to inappropriate immune responses within the gastrointestinal tract [34]. NOD1 is ubiquitously expressed, whereas NOD2 is expressed in hematopoietic cells. Both are cytosolic sensors that recognize bacterial

peptidoglycan motifs and activate NF- κ B signaling by interacting with several pathways using the RIPK2 adaptor (Fig. 1). NOD1 and NOD2 have been implicated in additional roles although the molecular signals and pathways triggering these functions are not known. These roles include induction of macroautophagy and regulation of adaptive immune responses [35, 36].

Other NLR proteins that are not known to incorporate into inflammasomes have also been implicated in antimicrobial function and immunity. NLRC5 has been reported to have an anti-inflammatory function via regulation of NF- κ B and type I IFN responses, and its absence enhances antiviral immunity [37, 38]. Both NLRP10 and NLRP12 may be anti-inflammatory and participate in dendritic cell migration and formation of an adequate adaptive immune response [6, 7, 9].

NLR proteins during infection with *Trypanosomatid* protozoa

The *Trypanosoma* spp. and *Leishmania* spp. protozoa (Order *Kinetoplastidae*, Family *Trypanosomatidae*) are vector-borne protozoan parasites which are pathogenic for humans and other mammals. *Trypanosoma cruzi* is a cause of American trypanosomiasis or Chagas' disease. In its acute form, Chagas' disease manifests as a self-limited febrile illness. The severe forms of disease ensue years to decades after initial infection in 30–40 % of infected persons, causing either chronic enlargement of the heart and heart failure or chronic enlargement of the esophagus and intestines. Both chronic disease forms are progressive and untreatable. The two subspecies of *Trypanosoma brucei* (*T. brucei rhodesiense*, *T. brucei gambiense*) cause African sleeping sickness (also called human African trypanosomiasis or HAT). More than 20 species of *Leishmania* cause a variety of human disease syndromes, the most common of which are local skin ulcers during cutaneous leishmaniasis, and visceral leishmaniasis associated with systemic spread of the parasite and chronic immunocompromise. The most important factor determining which clinical syndrome occurs is the infecting species of *Leishmania*.

Trypanosoma cruzi, NOD1, and NLRP3

Between seven and eight million people are infected with *T. cruzi* worldwide, and 30 % of chronically infected individuals will develop cardiac disease [39, 40]. Although it is unknown whether long-term chronic manifestations can be prevented by immune responses, murine models and severe infections in humans with immunocompromise have illuminated mechanisms by which the immune system contains the parasite. Immune control of the infection requires both innate and adaptive immune responses [41]. Innate immune responses implicated in parasite recognition include pattern recognition receptors TLR2, TLR4, TLR7, and TLR9 [42–44]. An inflammatory response to *T. cruzi* occurs only in hosts with intact MyD88 and ultimately drives the development of the adaptive response [45]. The NLR proteins NOD1 and the NLRP3/ASC/caspase-1 inflammasome also appear to be important in parasite sensing and inflammatory response [36, 46, 47].

One study implicates NOD1 in resistance to *T. cruzi* infection. This study of knockout mice lacking either NOD1 or NOD2 proteins showed that the absence of NOD1, but not NOD2, led to an exacerbation of *T. cruzi* parasite load and greater mortality. Although not as severe

as the defects in mice lacking either NOS2 or MyD88, the study linked the defect to poor response to IFN- γ in infected macrophages. The absence of NOD1 did not impair peripheral cytokine levels, implying the role of NOD1 in murine resistance to *T. cruzi* is independent of systemic cytokines [36].

The role of the NLRP3 inflammasome in *T. cruzi* infection has been described in two studies demonstrating that activation of the NLRP3 inflammasome by *T. cruzi* is protective in the mouse model of Chagas' diseases. The Silva group showed activation of inflammasome-related genes in the tissue of *T. cruzi*-infected mice and secretion of IL-1 β in infected murine macrophages. Furthermore, they showed that the secretion of IL-1 β after *T. cruzi* infection was dependent upon NLRP3, ASC, and caspase-1 using knockout mice and macrophages. In murine macrophages, this NLRP3 inflammasome activation was dependent upon K⁺ efflux, ROS production, and lysosomal damage. The authors posit that the exit of parasites from the macrophage phagosome to become cytosolic amastigotes releases damage-associated molecular patterns (DAMPs) that trigger NLRP3 inflammasome assembly, although this assertion remains to be demonstrated. Evidence that an active inflammasome was protective for mice infected with *T. cruzi* was derived from study of ASC and caspase-1 knockout mice, which developed more severe illness and higher parasite burdens in end organs compared to wild-type controls [47]. Another study using NLRP3 and caspase-1 knockout mice confirmed that murine infection with *T. cruzi* activates the NLRP3 inflammasome resulting in production of IL-1 β . This activation was protective; that is, mice lacking the NLRP3 inflammasome had increased parasite burdens. NLRP3-deficient macrophages were severely defective in killing *T. cruzi*, but surprisingly neutralization of IL-1 β or IL-18 did not abrogate the protective effects of NLRP3 inflammasome activation in vitro. Instead, nitric oxide production was severely compromised in infected NLRP3- and caspase-1-deficient macrophages compared to wild type. This caspase-1-dependent nitric oxide release was required to control macrophage infection with *T. cruzi*, as shown with the use of caspase-1 inhibitors. This may point to a novel cytokine-independent function of inflammasome-activated caspase-1 [46].

The above studies lead to the conclusion that the NLR protein NOD1, but not NOD2, partially protects against acute *T. cruzi* infection of mice. The NLRP3 inflammasome also plays a role in protecting against *T. cruzi* infection in a murine model. NLRP3-mediated control of *T. cruzi* was suggested to be through caspase-1-dependent NO production, but independent of caspase-1-induced IL-1 β /IL18 secretion. The mechanisms through which both of these NLR proteins control the parasite involve macrophage-mediated microbicidal responses, although the specific pathways need to be further elucidated.

***Leishmania* species and NLRP3**

Leishmania species parasites cause leishmaniasis, a group of diseases affecting approximately 0.2–0.4 million individuals with new cases of visceral leishmaniasis and 0.7–1.2 million persons with new cases of cutaneous leishmaniasis cases [48, 49]. The worldwide distribution includes 98 endemic countries [50]. Most cases of leishmaniasis are transmitted by a *Phlebotomus* spp. or *Lutzomyia* spp. sand fly. Parasites develop in the sand fly gut to virulent metacyclic promastigotes which are then inoculated into mammalian skin.

Promastigotes are rapidly taken up through receptor-mediated phagocytosis by local phagocytic cells such as macrophages, dendritic cells, or neutrophils, where they transform into the obligate intracellular amastigotes that replicate within the phagosome [51–54]. The *Leishmania spp.* parasites cause chronic infections whose symptoms range from cutaneous localized disease in which the pathology is caused by a vigorous inflammatory immune response, to a fatal visceral disease characterized by profound immunosuppression. Disease manifestations depend upon the species of parasite as well as the individual innate and adaptive immune response of the host [55, 56].

A role for NLR proteins is just beginning to be explored in models of the many species and varieties of leishmaniasis. Surprisingly little has been reported to date. The most studied model of leishmaniasis is the *L. major* model of cutaneous leishmaniasis. A notable characteristic of cutaneous leishmaniasis is the fact that “disease” symptoms are caused by a vigorous inflammatory response, which often but not always corresponds to the magnitude of parasite load in tissues surrounding a site of cutaneous ulceration. BALB/c mice are susceptible and fail to control the infection due to a predominant T helper 2-type (Th2-type) response to an immunodominant parasite antigen LACK [48]. This contrasts with resistant C57BL/6 mice which control the infection by mounting a T helper 1-type (Th1-type) immune response associated with IFN γ and IL-12 production [57]. Inflammation plays a central role in the pathogenesis of cutaneous leishmaniasis, but the role of cytokines produced by inflammasomes is not fully defined in models of cutaneous leishmaniasis. Two simultaneous reports of IL-18 in murine *L. major* infection documented a synergistic role for the cytokine in resistance to infection. One report found that IL-18 and IL-12 synergized in promoting resistance of BALB/c mice to pathology and protective immunity, and the other reported that the absence of IL-18, accomplished through either antibody-mediated depletion of BALB/c or genetic deletion in C57BL/6 mice, led to increased pathology [58]. The second group reported IL-18 plays a role only in protection early in *L. major* infection of IL-18 knockout C57BL/6 mice [59]. These reports suggest that, although IL-18 contributes to parasite control, it is not absolutely essential for protective immunity.

There is also an established role for IL-1 β in leishmaniasis. Two species causing cutaneous leishmaniasis, *L. major* and *L. amazonensis*, differ in that the former leads to self-curing infections in all but BALB/c mice, whereas the latter causes progressive non-curing infection. Xin et al. [60] attributed this difference to differential induction of dendritic cell maturation. In particular, murine dendritic cells infected with *L. amazonensis* were less mature and released less IL-1 β than those infected with *L. major*. Antigen presentation by dendritic cells led to a non-curative and inflammatory CD4⁺ T cell phenotype producing IL-10 and IL-17, as opposed to a curative Th1 response induced by dendritic cells infected with *L. major*. A subsequent study was conducted with knockout mice lacking IL-1 α , IL-1 β , or IL-1Ra (IL-1 receptor antagonist) on a susceptible BALB/c background. The authors reported that IL-1 α/β promotes the development of lesions due to *L. major* infection. Consistent with this, the lack of IL-1Ra, leading to unchecked IL-1 activity, leads to exacerbated parasite loads, and the authors suggest there is a divergence between disease and parasite load. [61]. Another study reported that *L. major* infection of IL-1 α/β knockout mice on a resistant C57BL/6 background led to a delay in *L. major* lesion resolution rather than an overall decrease in lesions, and double knockout DCs were fully capable of inducing a

protective immune response [62]. These results are further complicated because IL-1 α , which uses the same receptor as IL-1 β , is only partially dependent upon the inflammasome [63]. The effects of IL-1 α may be distinct from IL-1 β in leishmaniasis. IL-1 α administration was found to be protective in *L. major* infection in both susceptible [64] and resistant mice, although ablation of both IL-1 α and IL-1 β did not cause increased disease in resistant mice [62]. Overall, these results suggest that IL-1 contributes to the pathology of cutaneous leishmaniasis and may also contribute to parasite control in some models. Nonetheless at this juncture, there is an unclear definition of the full role of IL-1 β in murine models of cutaneous leishmaniasis, as these results could implicate different functions of IL-1 β depending on the inoculum dose, innate resistance of the host model, and the species of *Leishmania*.

There has been one report indicating that at least one *Leishmania* species is capable of activating the NLRP3 inflammasome in macrophages in vitro, and in vivo evidence that NLRP3 inflammasomes influence the course of murine cutaneous or visceral disease. Knockout mice lacking NLRP3, ASC, or caspase-1 developed increased lesion size and increased parasite burden compared to wild-type control mice when infected with *L. amazonensis*. There was evidence that NLRP3 and inflammasome components limit the intracellular growth of *L. amazonensis* in macrophages. However, conflicting internal data in this report showing a decrease in IL-1 β and caspase-1 cleavage in infected macrophages led to confusion about the exact role of the classical NLRP3 inflammasome. Nonetheless, in vitro data demonstrated that IL-1 β release by macrophages infected with *L. amazonensis*, *L. braziliensis*, and *L. mexicana* required macrophages expressing NLRP3, ASC, and caspase-1, and an in vivo role for NLRP3 was shown for *L. infantum* [65]. Although not all data were consistent with direct activation of inflammasomes by parasite products, the study raises the very intriguing possibility that the NLRP3 inflammasome may become activated and provide an essential role in resistance to at least some *Leishmania* species, particularly *L. amazonensis* [65]. Careful comparison with other species is essential because of the very different pathogenesis and intracellular compartmentalization of *L. amazonensis* compared to other *Leishmania* species [66, 67].

Overall, these experiments suggest a protective role for the NLRP3 inflammasome against *Leishmania* parasite load, with increased host resistance to a high parasite load. There is conflicting evidence suggesting that inflammasome assembly or IL-1 β could actually exacerbate the size of lesions of cutaneous leishmaniasis. The underlying mechanisms need further exploration, as does the universal nature of these mechanisms to other *Leishmania* species. Little is known about any putative role of the NOD1/2 proteins in leishmaniasis. Similarly, nothing is yet reported about the roles of the “non-inflammasome” NLRs in leishmaniasis, although their importance in inflammation, and developing adaptive immunity, suggests these pathways could be important.

Apicomplexan protozoa

Malaria, NLRP3, and NLRP12

Malaria is a fatal parasitic disease transmitted to humans by mosquitoes carrying one of the five species of *Plasmodium*. About 3.3 billion people, or half the world's population, live in

Studies in another murine model of cerebral malaria due to *P. chaboudi adami* DS corroborated a role for the NLRP3 inflammasome in the pathogenesis of cerebral malaria. Using knockout mice, the investigators showed Hz-induced IL-1 β activation release was dependent upon the presence of NLRP3, ASC, and caspase-1 in murine bone marrow macrophages. Lack of each of these improved survival of each of these knockout mouse strains upon infection with *P. chaboudi adami*. Furthermore, this study demonstrated that the tyrosine kinases Lyn and Syk were needed to activate the NLRP3 inflammasome [80]. Interestingly, another study of the *P. berghei* ANKA model reported that NLRP3 knockout mice had delayed onset of cerebral malaria, but mice lacking ASC, caspase-1, or IL-1RI had unchanged survival, leading to the suggestion that there may be a role for NLRP3 independent of the inflammasome [81]. Yet another contrasting report showed that murine *P. berghei* ANKA cerebral malaria was not altered in the absence of caspase-1, IL-1 β , or IL-18 when infection was initiated with sporozoites rather than infected erythrocytes and that pathology was solely dependent on host TLR2/4 and MYD88. As such, the IL-1 β inhibitor Anakinra was not protective against cerebral malaria in this model [82]. The difference between these studies and outcomes is not entirely evident, although it is possible that experimental factors (infecting parasite species and stage, subtle differences in protocol leading to more Hz–DNA complex in one model than another) could play a role and/or that there is indeed an inflammasome-independent role for NLRP3.

In contrast to the exacerbatory effect of the NLRP3 inflammasome on cerebral malaria, other investigators demonstrated that there is an adjuvant effect of Hz when administered with vaccine against *P. falciparum*, and this activity was entirely dependent upon MyD88 and not the NLRP3 inflammasome [83].

Studies of human immune responses during malaria confirm a putative role for inflammasomes. Human primary peripheral blood cells incubated with *P. falciparum* confirmed that the inflammasome is activated and cytokines are secreted by human macrophages in response to Fc γ R-mediated phagocytosis of infected RBCs. Inflammasome activation was confirmed by cleavage of caspase-1 and production of IL-1 β , both of which were abrogated by caspase-1 inhibition [84]. Overlap or synergy between TLR- and NLRP3-induced inflammasome responses during the innate immune response to malaria is likely. A study of both human and murine cells showed that *P. falciparum* or *P. berghei* ANKA malaria hemozoin–DNA complexes activate TLR9 in human or murine macrophages, respectively. Injection of infected erythrocytes into mice also resulted in inflammasome activation and release of IL-1 β . Parallel experiments with gene knockout mouse erythrocytes infected with *P. berghei* ANKA revealed that both the NLRP3 inflammasome responding to Hz and the AIM2 inflammasome responding to *Plasmodium* DNA are activated and produce IL-1 β [26].

A recent murine study suggests that both NLRP12 and NLRP3 promote inflammasome formation in response to *Plasmodium chabaudi* AS infection with bacterial superinfection. Wild-type mice infected with *P. chabaudi* produced high levels of IL-1 β in response to a secondary challenge with bacteria or LPS, and mice superinfected with bacteria succumbed to septic shock. In contrast, knockout mice lacking ASC, caspase-1, NLRP3, or NLRP12 had improved survival during a parallel bacterial superinfection. The authors proposed a

dual role for NLRP3 and NLRP12 in forming inflammasomes during dual malarial–bacterial infection [85]. However, they stopped short of showing that NLRP12 is incorporated into inflammasomes and did not distinguish between responses to the parasite alone versus the parasite and bacteria. Since NLRP12 has been reported by other investigators to be anti-inflammatory through mechanisms that do not involve inflammasomes [8, 9], this model warrants revisiting.

Overall, the above studies suggest a role for NLRP3-induced inflammasomes and for TLR9 in the response to malaria hemozoin with or without complexed malaria DNA. They also implicate TLR9 in the pathologic responses to cerebral malaria in murine models. Potential involvement of AIM2-induced inflammasomes and the role of NLRP12 will require further investigation. It remains to be seen whether the NLR proteins play a role in exacerbating human malarial symptoms, including the deadly complication of cerebral malaria.

Toxoplasma, NOD2, NLRP1, and NLRP3

Toxoplasma species infect a broad host range of animals and birds including humans. *T. gondii* infection rates in humans are as high as 30–50 % worldwide [86]. The parasite is transmitted both horizontally and vertically in humans. Most immune competent human hosts are asymptomatic during toxoplasmosis, although occasionally a mononucleosis-like syndrome can occur. Immune-compromised individuals, however, can develop serious disease symptoms due to toxoplasmosis, including encephalitis and organ failure. Some of the severe conditions are congenital toxoplasmosis and CNS toxoplasmosis [87].

Toxoplasma gondii trophozoites can infect any nucleated cell. Robust cellular immunity, including inflammatory monocytes, a Th1 adaptive immune response and an NK cell response are needed to control infection [41, 45, 88, 89]. The importance of NOD2 in forming a protective Th1 immune response during toxoplasmosis was shown in a study utilizing NOD2 knockout mice. NOD2-deficient mice are unable to clear *T. gondii* and fail to mount an appropriate adaptive response. Dendritic cells are fully functional, and the NOD2 defect in immunity was shown to be due to a T cell intrinsic defect with impaired nuclear localization of c-Rel and defective *Ii2* transcription [90]. A search for genetic factors that influence susceptibility to toxoplasmosis led to even more exciting results. Using case–parent trios and transmission disequilibrium testing, human susceptibility to congenital toxoplasmosis was found to be significantly associated with SNPs in the *NLRP1* gene locus. The role of NLRP1 in toxoplasmosis was investigated using RNAi knockdown of NLRP1 in a transfected human cell line. This revealed that *Toxoplasma* infection of cells with NLRP1 knockdown failed to induce production of inflammatory cytokines including IL-1 β , IL-18, and IL-12 compared to control cells. NLRP1 knockdown cells also underwent accelerated cell death upon *T. gondii* infection [90, 91]. Using a murine model, Boothroyd’s group showed the NLRP1b inflammasome was activated during *T. gondii* infection of mouse and rat models, leading to protective immunity against oral challenge infection [92]. The parasite “ligand” for the inflammasome forming NLR, presumably NLRP1, was clearly shown in human monocytes or a human cell line infected with *T. gondii*. In this study, IL-1 β release was dependent on mammalian cell caspase-1 and ASC, and on the parasite protein GRA15, the presumed agonist for NLRP1 [93]. Recently, Grigg’s group implicated the NLRP3

inflammasome as well as the NLRP1 inflammasome in murine resistance to *T. gondii* infection. In vivo, *T. gondii* activated the NLRP3 inflammasome in macrophages. Mice lacking NLRP3, ASC, and NLRP1 all displayed increased parasite burden and mortality. These studies further indicated that IL-18 may be a key cytokine in inflammasome-mediated resistance to *T. gondii* infection [94].

Thus, in contrast to malaria, NLRP1 and NLRP3 inflammasome activation induced by *T. gondii* infection limits the parasite load and dissemination. The role of NOD2 seems to be important as a sensor necessary for development of an effective adaptive response to *Toxoplasma*.

Schistosomes and NLRP3

The parasites discussed so far are unicellular protozoa, most of which can become intracellular and interact with NLRs or other intracellular host microbicidal pathways. In contrast, the *Schistosoma* species are a group of multicellular helminthic parasites which live in the human host as intravascular adult worms releasing inflammatory eggs into tissues. Schistosomiasis is transmitted in fresh bodies of water that are contaminated with human excrement, and which support growth of the intermediate snail host. Parasites directly penetrate through human skin and become a larval form before converting to adult worms. Eggs released by these adults not only can be released back into the environment to complete the life cycle, but also they can enter tissues causing a granulomatous response and severe inflammatory disease. More than 240 million people are infected with schistosomiasis worldwide, and more than 700 million are living in endemic areas [95]. As they are not intracellular, it is not immediately evident how these parasites would interact with the NLR proteins.

Among the species known to infect humans, a common parasite causing gastrointestinal or hepatic schistosomiasis is *S. mansoni*. Murine models show the immune response is characterized by a strong initial Th1 response to inoculated parasites, which later switches to a strong Th2 response to schistosomal egg antigen. Pathologic responses in *S. mansoni* disease are secondary to T-cell granulomas in the liver and intestines in response to eggs that become embedded in the tissue [96, 97]. It has previously been reported that components of soluble egg antigen (SEA) suppress TLR9-mediated dendritic cell response and release of TNF- α and IL-6 [98]. The da Costa group extended this observation to note that SEA dampens signaling through TLR7, TLR8, and TLR9, while simultaneously activating the NLRP3 inflammasome and triggering IL-1 β secretion from mouse macrophages. Cells from knockout animals showed that this activation is dependent upon caspase-1 and NLRP3. The mechanism of activation was through an SEA component binding to Dectin-2, which associates with the Fc γ chain, activating Syk kinase and leading to ROS production and K⁺ efflux. Interestingly, however, during *S. mansoni* infection of knockout mice lacking either NLRP3 or ASC yielded a milder disease and decreased Th1, Th2, and Th17 cytokine production compared to wild-type mice [99].

Schistosomiasis is another example in which the inflammasome seems to promote pathology as opposed to cure or prevention of parasitic disease. Even though SEA triggers NLRP3 and

IL-1 β secretion leading to immunopathology in the liver, SEA may also be important for production of antigen-specific adaptive immune response.

Conclusions

Parasitic pathogens affect large segments of the global population and result in significant morbidity and mortality worldwide, yet are comparatively less studied than bacterial and viral pathogens. For this reason, many parasitic diseases are included in the list of “neglected tropical diseases,” including malaria, Chagas’ disease, leishmaniasis, and schistosomiasis [40, 50, 69, 95]. The host immune response and the parasites’ manipulation of that response are key areas of study that must be understood to achieve the goals of developing safe and effective vaccines and treatment regimens to control these important diseases.

The NLR proteins are crucial in sensing and responding to pathogens. Their functions in parasitic diseases are beginning to be described, particularly NOD1, NOD2, and the inflammasome forming NLRP3 and NLRP1 molecules. The NLR protein NOD1 is protective in *T. cruzi* infection, NOD1 and NOD2 function in inflammatory cytokine production in cerebral malaria, and NOD2 may be necessary to developing a T cell response to *T. gondii*. The NLRP1 and NLRP3 inflammasomes act as essential sensors of *T. gondii* and cause inflammation but are needed to limit parasite burden and dissemination. The NLRP3 inflammasome is protective to the host in *T. cruzi* infections, controlling parasite burdens and limiting end organ damage and may also be protective against *Leishmania* parasite burden, although it may also contributed to the inflammation that occurs during cutaneous leishmaniasis. The NLRP3 inflammasome, and possibly the AIM2 inflammasome, may increase pathogenesis and decrease survival in at least some models of cerebral malaria. NLRP3 causes immunopathology in the liver in response to *S. mansoni* egg antigens but may also be important for production of an adaptive immune response.

The investigation of NLR proteins in parasitic disease models is shedding light on some hitherto unknown functions and new mechanisms of actions of these proteins. For example, the functions of both NOD1 and NLRP3 in the *T. cruzi* mouse model appear to be novel, with NOD1 acting independent of NOD2 and the NLRP3 inflammasome acting primarily through enhanced nitric oxide production rather than through IL-1 β [36, 46]. The great evolutionary diversity of parasitic disease-causing organisms is a strong argument for investigating NLR proteins in the broadest array of parasitic pathogens, as there will likely be unexpected interactions and evasion mechanisms that can shed light on the mechanisms by which the host senses pathogens and invokes inflammatory pathways.

Some NLR proteins may be needed for the host response to parasite infection by acting as innate immune sensors and driving the inflammatory response. However, the inflammatory response driven by these same proteins can also result in pathology during some parasitic diseases. Unregulated NLR-induced pathways can lead to organ failure and death. Dissecting the roles of NLR proteins in host defense and inflammatory pathology due to parasitic diseases will be invaluable background for the development of treatments and vaccines that must balance the roles of inflammation in protective immunity versus inflammatory injury.

Acknowledgments

This work was supported in part by Grants R01 AI076233 (MEW) and NIH R01 AI087630 (F.S.S.) from the National Institutes of Health and by grants 1i01BX001983 and 5I01BX000536 from the Department of Veterans' Affairs.

Biography



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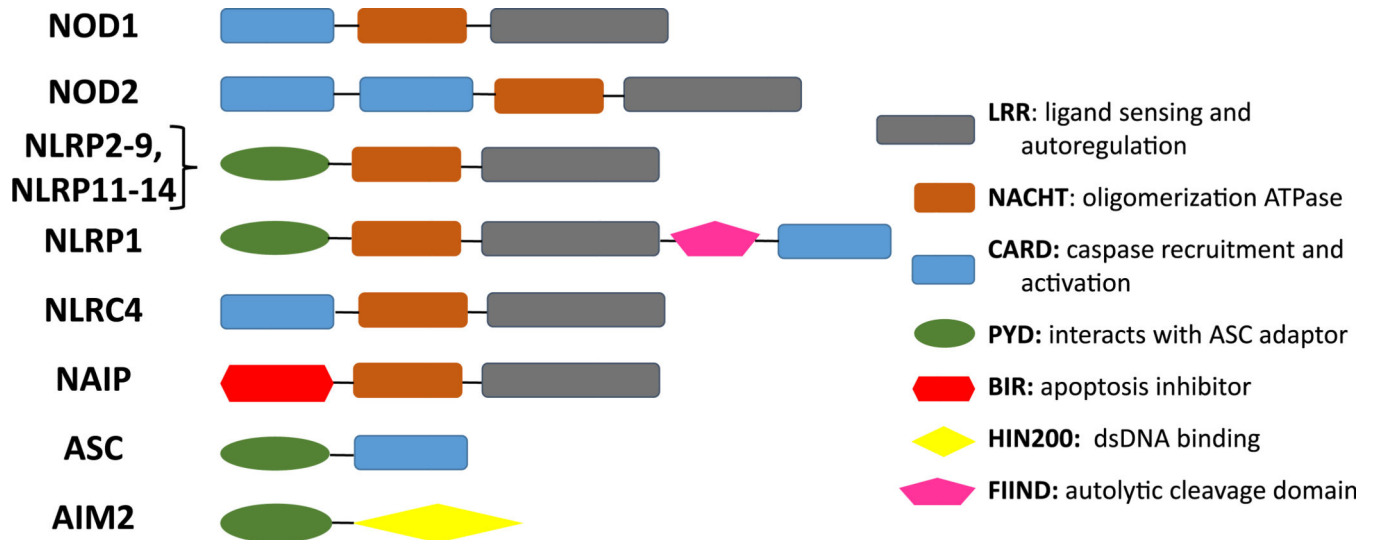


Fig. 1.
NLR and related protein domains

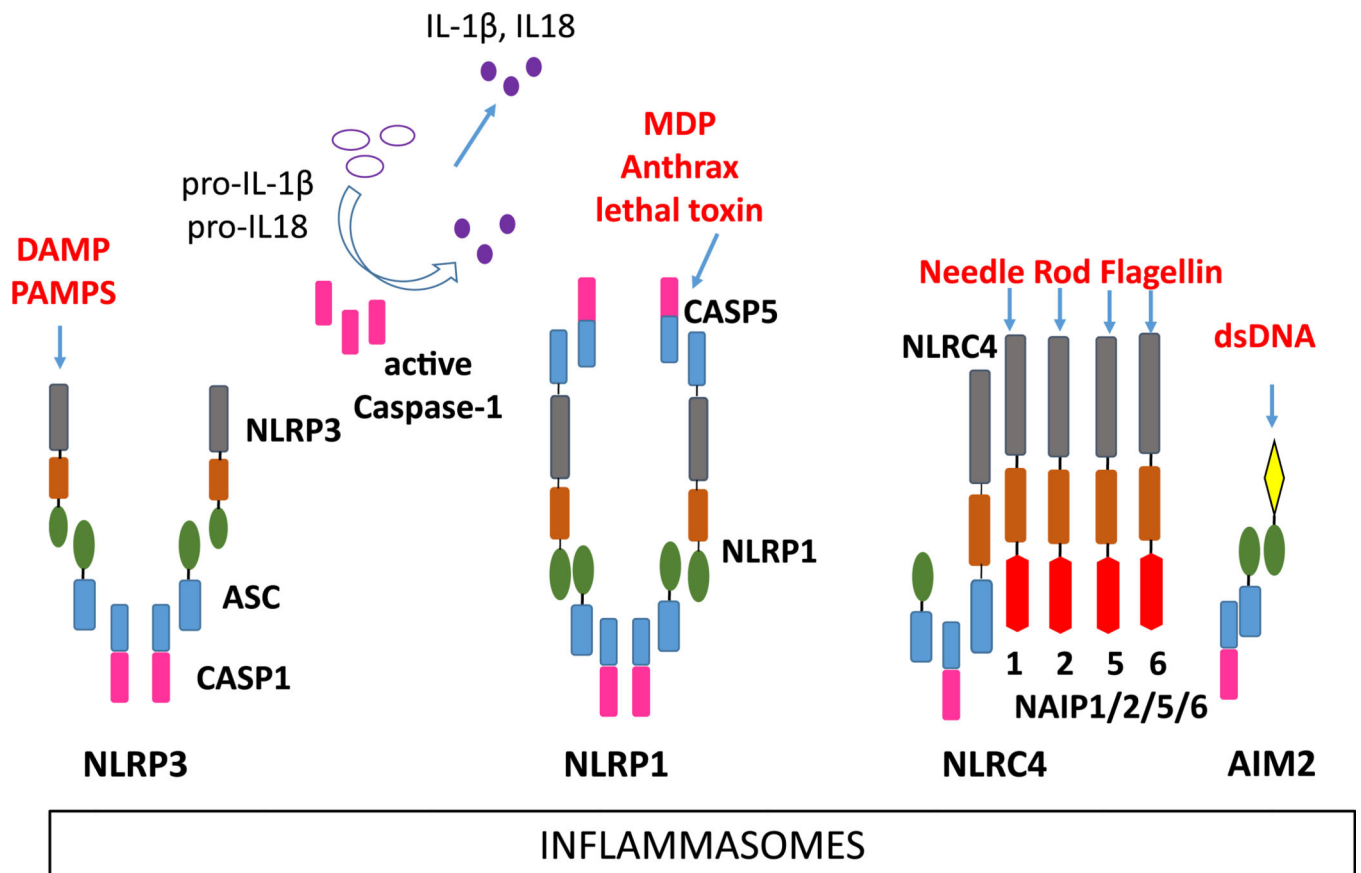


Fig. 2. Inflammasome assembly. *Left to right* NLRP3 inflammasome with the ASC adaptor and caspase-1; NLRP1 inflammasome assembly with ASC, and caspase-1 or caspase-5; NLRC4 inflammasome with NAIP1, NAIP2, NAIP5, or NAIP6, ASC (the human NAIP is equivalent to the murine NAIP1) and caspase-1; AIM2 inflammasome with the ASC adaptor and caspase-1