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Authors

Kurabi, Arwa
Pak, Kwang
Ryan, Allen F
et al.

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Innate Immunity: Orchestrating Inflammation and Resolution of Otitis Media

Arwa Kurabi[1,3] · Kwang Pak[1,3] · Allen F. Ryan[1,3] · Stephen I. Wasserman[2]

Abstract Otitis media (OM) is a common disease in young children, accounting for more office visits and surgeries than any other pediatric condition. It is associated with an estimated cost of five billion dollars annually in the USA. Moreover, chronic and recurrent middle ear (ME) disease leads to hearing loss during critical periods of language acquisition and learning leading to delays in reaching developmental milestones and risking permanent damage to the ME and inner ear in severe cases. Therefore, research to understand the disease pathogenesis and identify new therapeutics is important. Although OM is a multifactorial disease, targeting the molecular mechanisms that drive inflammation and OM resolution is critical. In this review, we discuss the current evidence suggesting that innate immune receptors and effectors play key roles in OM by mediating both the ME inflammatory responses and recovery.

Keywords TLR · IL-1ß · Innate immunity · Inflammasome · Middle ear · Inflammation

✉ Arwa Kurabi
   akurabi@ucsd.edu

   Kwang Pak
   kpak@ucsd.edu

   Allen F. Ryan
   afryan@ucsd.edu

   Stephen I. Wasserman
   swasserman@ucsd.edu

1   School of Medicine, Department of Surgery, Division of Otolaryngology, University of California San Diego, 9500 Gilman Drive—MC#0666, La Jolla, CA 92093-0666, USA

2   School of Medicine—Department of Medicine, Rheumatology, Allergy and Immunology Division, University of California San Diego, 9500 Gilman Drive—MC#0628, La Jolla, CA 92093-0628, USA

3   Veterans Administration San Diego Healthcare System, 3350 Villa La Jolla Drive—MC#151, San Diego, CA 92121, USA

Introduction

Otitis media (OM) is a common infectious disease in children worldwide, resulting in substantial health care expenditures and burden [1, 2]. In the USA, it is the most common condition warranting medical therapy for children under 5. More than 90 % of children experience OM before age 5 [3, 4]. While acute OM (AOM) tends to be uncomplicated and self-limiting, 10–20 % of children experience persistent, recurrent, or chronic OM [5]. The long-lasting forms of this condition can result in hearing loss, delayed speech and communication development, and may carry a risk of permanent damage to the middle and inner ear resulting in deafness, with more serious complications in developing countries [6–8]. Currently, treatment for uncomplicated OM consists of watchful waiting or antibiotics [9, 10]. Tympanostomy tubes for middle ear (ME) ventilation are often recommended for recalcitrant cases [11–13]. The insertion of tympanostomy tubes is the most common ambulatory surgery performed on children in the USA, at 670,000 insertions annually costing over 4 billion dollars [14].

The etiology of OM is multifactorial. OM incidence can be influenced by infectious pathogen variations, host anatomy, and immunological status. At the molecular level, OM is defined by a ME inflammatory response as a result of the activation of pro-inflammatory transcription factors followed by

the production and release of inflammatory cytokines, mucosal hyperplasia, leukocytic infiltration into the ME cavity, and secretion of mucus-rich effusions [15••], all aiding in microbial clearance. Nonetheless, there is evidence that indicates that bacteria can form highly organized and well-structured biofilms in the ME, thereby evading immune clearance by the host [16] and prolonging pathogenesis. *Streptococcus pneumonia*, nontypeable *Haemophilus influenza* (NTHi), and *Moraxella catarrhalis* are the top three common pathogens causing OM [5, 17]. However, OM is often preceded by respiratory viral infections, and viruses are often detected in ME effusions [18, 19]. Vaccines against pneumococcal conjugates (PVCs) have led to decreases in the prevalence of *S. pneumonia* serotypes detected during OM [20–22]. However, increases in OM cases due to other pathogens have increased [23]. Children are more prone to OM than adults for several reasons. Their Eustachian tube is shorter, oriented differently, and functions less efficiently compared to adults, allowing easier bacterial access to the ME from the nasopharynx [24, 25]. In addition, they are immunologically naïve to OM pathogens and their immune systems are immature [26, 27]. Indeed, only a subset of 10–20 % exhibits recurrent or chronic disease. Children who experience more than three episodes of AOM within 6 months are considered otitis-prone, and are likely to require tympanostomy tube insertion [11, 28].

The causes of persistent ME infections and the reasons why some children progress to persistent/recurrent OM while others experience no or fewer OM episodes are not fully understood. Epidemiologic studies indicate that OM proneness in humans receives contributions from infection-related Eustachian tube dysfunction, immunologic naïveté, economic and health care status, plus prior exposure to upper respiratory viral infections [13, 17, 29]. However, it is also clear that genetics play a significant role, as indicated by twin studies [29–32]. OM proneness is almost certainly polygenic, and there is evidence associated between genes involved in craniofacial structure as well as in immune defense [24, 33]. Craniofacial anomalies likely disrupt the normal function of the Eustachian tube, leading to altered ME pressure as well as access of bacteria to the tympanic cavity [8]. However, the great majority of children with chronic/recurrent OM do not have overt craniofacial abnormalities. Regarding immunity, in general, there are two distinct defense strategies that can protect and restore a host from infection: (i) alleviating the pathogenic burden by increasing host resistance and (ii) reducing the immunopathological impact of infection by raising host tolerance [34••, 35]. Changes in these two fundamental defense mechanisms (host tolerance or host resistance), which often contribute to other forms of chronic inflammatory diseases, can also be linked to OM proneness. These include mutations or polymorphisms in genes that subserve innate immunity, defects in cellular processes that regulate infection such as phagocytosis, and the dysfunction of cellular and other

factors that initiate and regulate tissue repair and recovery after inflammation and injury. A recent survey of the transcriptome of otitis-prone children with NTHi AOM identified how many innate immune genes and genes related to the inflammatory responses are altered and/or downregulated [36•].

Innate Immunity and OM

In the normal child, uncomplicated AOM resolves in only a few days, even in the absence of antibiotic therapy [3]. This period is too short for the development of cognate immunity to play a significant role in the resolution of infection. This implicates the innate immune system, which is activated without prior sensitization, as the major effector of OM resolution. Figure 1 provides a schematic overview of several of the innate sensing receptors that have been predicted to play a role in OM.

Over the past two decades, many fundamental discoveries have been made regarding the mechanisms by which ME infection is recognized by innate immune sensors and contained by the responses of the innate immune system [15••]. Much of this progress has been accomplished using animal models to understand the etiology, pathophysiology, and recovery processes of OM [37, 38•, 39•, 40, 41]. A summary of published studies that utilize the mouse model system to study the role of innate immunity and related receptors in OM development and recovery is shown in Table 1.

The innate immune system is comprised of pattern recognition receptors (PRRs) that respond to pathogen-associated molecular patterns (PAMPs). These include the Toll-like receptors (TLRs), Nod-like receptors (NLRs), Rig-like receptors (RLRs), C-type lectin receptors (CLRs), and DNA receptors [56, 57]. The activation of these receptors, which can be either extracellular or cytoplasmic by pathogen molecules, results in the initiation of inflammation and other mechanisms critical not only for the clearance of invading microorganisms and the restoration of tissue homeostasis but also for the activation and sensitization of the adaptive immune system. Activated PRRs recruit adaptor molecules, which in turn initiate signaling cascades. The majority of these cascades end in the activation of transcription factors, including NFκB, AP-1, and IRFs, which localize to the nucleus and activate a broad array of genes involved in host defense [58]. This includes genes encoding cytokines and chemokines that recruit and activate leukocytes including neutrophils, monocytes, macrophages, and NK cells. Infected epithelial cells become targets of NK cells, while neutrophils and macrophages aid in the phagocytic clearance of bacterial pathogens and dead cells [59].

In this review, we discuss how the activation of innate immune signaling pathways, in particular by the TLRs and NLRs, may be a common pathway for OM pathogenesis and recovery. The expression of PRRs in the ME has been examined by Granath et al. [60] and others [29, 61, 62]. Several are expressed
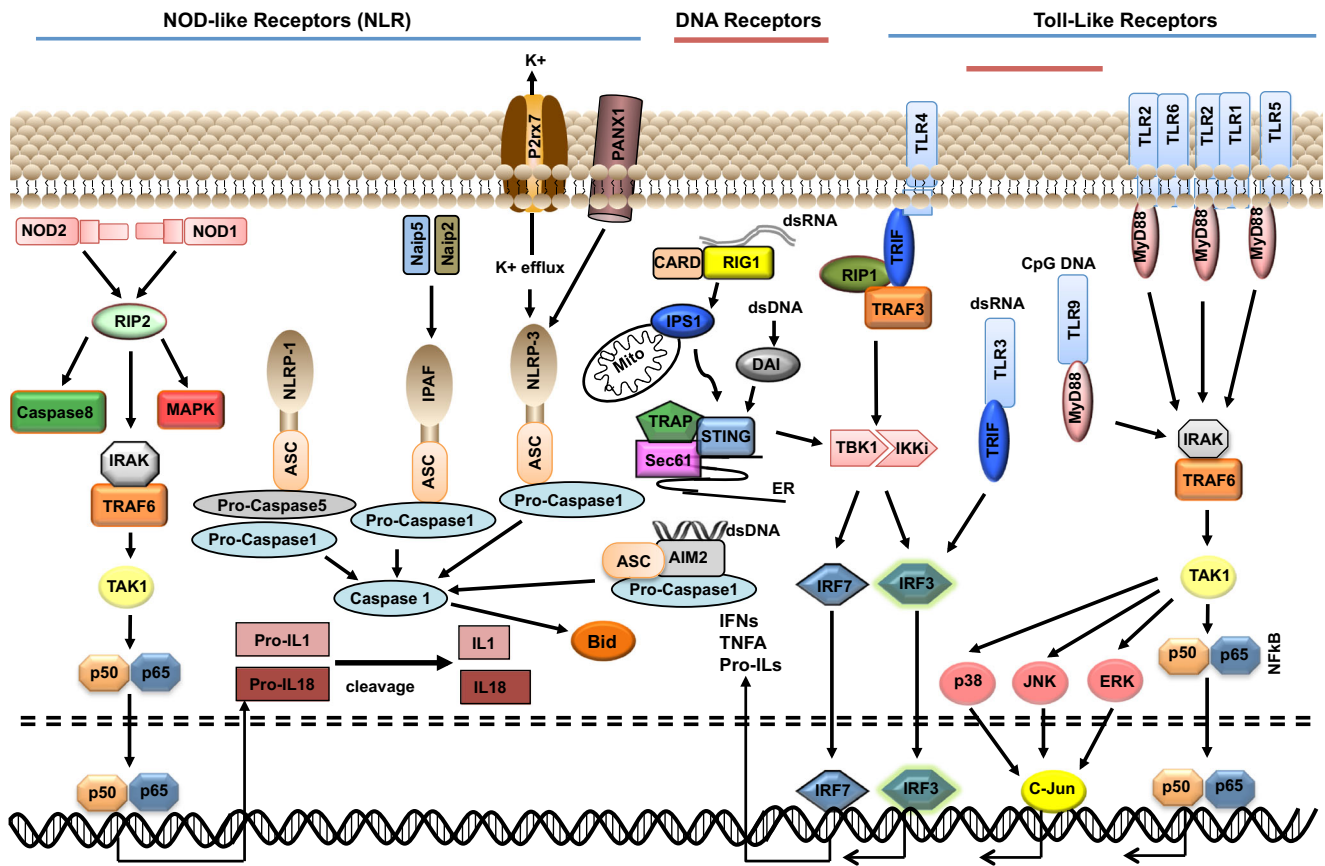
Fig. 1 Different innate immune signaling sensors implicated in OM. Toll-like receptors (TLRs) are membrane proteins that signal through either a MyD88-dependent inflammatory cytokine response and/or a TRIF (Tir-domain-containing adaptor inducing interferon β)-dependent type-1 interferon response (IRF). The NOD-like receptors (NLRs) organize into the large complexes known as inflammasomes which activate and release IL (interleukin)-1β and IL-18. Other members of the NLR family (NOD1 and NOD2) upon recognition of bacterial peptidoglycans self oligomerize into large structures and recruit the scaffold protein RIP2 (receptor interacting protein 2) which mediates a TAK1 (TGFβ activated kinase) activation of MAP kinase (MAPK), p38, JNK (c-Jun N-terminal kinase), and NFκB (nuclear factor κB) among other transcription factors. DNA sensors represent another class of innate immune receptors that can recognize bacterial and viral nucleic acid particles triggering an inflammatory response. These innate immune sensors regulate which transcription factors are activated, that in turn modulate the expression of pro-inflammatory and anti-inflammatory genes that regulate the host inflammatory response and healing

at significant levels in the normal ME cavity and, following infection, these and many additional PRRs are upregulated

Table 1 Current mouse models of OM related to innate immunity

Pathway	Gene	Background strain	References
Toll-like receptors	MyD88	C57 BL/6	[42]
	TLR 2	C57 BL/6	[43–45]
	TLR 4	C57 BL/6, C3H/HeJ	[43, 46]
	TLR 9	C57 BL/6	[47•]
TNF	TNF-α	C57 BL/6	[48, 49]
	TRIF	C57 BL/6	[50]
NLRs	ASC	C57 BL/6	[51]
	NOD2	C57 BL/6	[52]
Others	IL10	C57 BL/6	[53]
	JNK1/JNK-2	C57 BL/6	[54]
	TGIF	C57 BL/6	[55•]

significantly. Triggering of these PRRs activates the inflammatory immune responses that lead the efficient destruction of invading pathogens. The innate immune response is balanced between pro-inflammatory responses that fight infection and anti-inflammatory responses protecting against host tissue damage and initiating repair and healing. For example, NFκB induces both pro-inflammatory genes and genes that limit the duration and magnitude of the inflammatory response. Innate immunity also plays a critical role in the development of cognate immunity by recruiting and activating lymphocytes, as well as macrophages and other cells involved in antigen presentation. Thus, innate immunity affects not only initial defense against infection, but also the development of immunologic memory [63].

The TLR Family

TLRs were the first PRRs to be described in depth. There are 10 TLRs identified in humans, and 13 in mice [64]. The TLRs

have evolved to recognize non-host PAMPs from fungi, bacteria, viruses, and other parasites [59]. TLR1, TLR2, TLR4, TLR5, and TLR6 are cell surface receptors that primarily recognize lipoproteins, lipopolysaccharides (LPS), and flagellins in the extracellular environment. In contrast, TLR3, TLR7, TLR8, and TLR9 are cytosolic receptors located on endolysosomes and they primarily detect microbial nucleic acids [58]. Activated TLRs recruit and activate adaptor molecules, which initiate and amplify downstream signaling cascades. Most TLRs utilize the adaptor myeloid differentiation factor-88 (MyD88) [65]. On the other hand, TLR3 uses the adaptor protein TRIF, while TLR4 can utilize both the MyD88-dependent or TRIF-dependent pathways [57]. The MyD88 activation of the downstream IRAK1/4 (interleukin-1 receptor-associated kinase 1/4) scaffold results in the phosphorylation and binding of TRAF6. A resulting cascade activates the effector molecule TAK1, which in turn activates the canonical NFκB pathway. TAK1 can also activate the MAP kinases such as JNK, p38, and ERK (extracellular signal-regulated kinase) [66]. TRIF can activate not only TRAF6 (TNF receptor-associated factor 6), but also TBK1 (tank-binding kinase-1) leading to IRF activation. Subsequent pro-inflammatory responses mediated primarily by cytokines, chemokines, and interferons (IFNs) follow. However, TLR signaling pathways can be negatively regulated by negative feedback loops and anti-inflammatory cytokines like IL-10, TGFβ (transforming growth factor-β), and IL-1 receptor agonists [67]. Among the TLR subtypes, TLR2 and TLR4 play a crucial role in deciding the ultimate outcome of infection during OM [42–44, 68, 69]. TLR2 is involved in the recognition of a wide variety of microbial ligands like peptidoglycans, lipoteichoic acid, and lipoproteins. On the other hand, TLR4 primarily binds to LPS found in abundance on gram-negative bacteria.

Studies in animal models have demonstrated the crucial role of TLRs and TLR-dependent signaling cascades in the resolution of OM [44, 46, 70]. TLR mutations caused prolongation of OM in mice, while both TLR2- and TLR4-deficient mice exhibit persistent inflammation with impaired bacterial clearance and delays in OM recovery. In addition, genetic deletions of downstream signaling molecules, MyD88, TRIF, TNF (tumor necrosis factor), JNK1, and JNK2 also result in abnormal OM recovery [50, 54, 71]. In particular, mouse model studies have shown that in the absence of TLR2 or MyD88, initial neutrophil and macrophage recruitments are significantly delayed (decreasing host resistance), which lead to prolonged inflammatory response and the hosts inability to clear bacteria for long periods (up to 42 days, as compared to 5 days in wild-type mice). In particular, TLR2-deficient mice were susceptible to *S. pneumoniae* infection of the ME and showed decreased expression of NOD2, IL-1, NFκB, TNF-α, MIP1α (macrophage inflammatory protein 1α), Muc5ac (mucin 5, subtypes A and C), and Muc5b, encumbering timely bacterial clearance [45]. Furthermore, TLR4 induces the early activation of TLR2 in OM

[42] and plays a role in acquired adaptive mucosal immunity in the ME [43]. It appears that both TLR2 and TLR4 are important to induce an upregulation of the key pro-inflammatory mediator TNF-α early in OM [69]. This TLR-TLR interplay appears to induce the downstream target cytokines like CCL3 promoting macrophage recruitment and bacterial phagocytosis and clearance [48]. However, as mentioned previously, feedback regulators and anti-inflammatory factors tightly regulate these pro-inflammatory signals. It is thought that TGFβ inhibits MyD88-dependent TLR signaling pathway [72] and suppresses inflammatory genes such as iNOS (nitric-oxide synthase) via the Smad3 pathway [73]. Several mouse models implicating the anti-inflammatory TGFβ signaling in susceptibility to chronic OM have been reported to date [55•, 74, 75]. In addition, IL-10, which plays an anti-inflammatory factor role by inhibiting the NFκB-dependent pathway downstream of the TLRs, has been shown to regulate the ME mucosal hyperplastic immune response [53] via TLR2. Hence, IL-10$^{-/-}$ mice show a reduced number of goblet and mucin-producing cells during OM.

In humans, polymorphisms in genes encoding TLR2, TLR4, and the TLR4-binding partner CD14, have demonstrated a possible association with OM susceptibility [76], as have genes for the TLR effectors TNF-α and IL-1R (reviewed in [77]). Changes in the mRNA levels of TLR2, TLR4, TLR5, TLR7, and TLR9 in addition to other immune-associated genes, cytokines like IL-1, IL-6, IL-8, and IL-10 and chemokines like CCL2, CCL3, and CXCR3 (Chemokine (C-X-C Motif) Receptor 3) in the ME infiltrate and inflamed mucosa in patients have also been observed clinically [78–82]. The elevated levels of IL-10 were observed in the ME effusions and sera of children with OM [83]. Taken together with the animal data, these results provide strong evidence that the TLRs via innate immunity play a significant role in OM recovery.

The NLR Family

Another family of PRRs is the NLRs, which are receptors that detect bacterial and viral molecules in the cytoplasm, leading to the secretion of pro-inflammatory cytokines like IL-1β. The members of this family include NOD1 and NOD2, as well as the pyrins (NLRPs), and NLR family CARD (caspase recruitment domain) domain-containing protein 4 (NLRC4). The NLRs organize large signaling complexes such as Nod signalosomes and NALP (NACHT leucine rich repeat (LRR) and pyrin domain (PYD) containing 3) inflammasomes [84]. The activation of the NODs leads to the production of cytokines and chemokines via the adaptor RIP2, similar to the TLR/MyD88 cascade, ending in the activation of NFκB and MAPK pathways [85]. Alternatively, they can interact with mitochondrial antiviral signaling protein (MAVS), leading to type I interferon production [86]. Recent studies in patients with chronic OM with effusion found that the expression of

NOD1 and NOD2 mRNA was lower in the ME effusions of otitis-prone than in the non-otitis-prone individuals [62]. However, chronically inflamed mucosal tissue samples from patients with chronic OM revealed that NOD2 expression is upregulated in the ME mucosa compared to normal [81]. These increased expression levels of mucosal NOD2 in particular have been shown to play a role in regulating the NTHi induced β-defensin2 production and in turn modulate the recruitment of inflammatory cells and bacterial clearance in the ME cavity of NOD2$^{-/-}$ knockout mice [52], thus highlighting the interplay between leukocyte infiltrate response and the mucosal immune response in the ME.

The activation of the NLRPs results in the formation of inflammasomes, a large multi-molecular signaling platform consisting of an active NLRP, the bipartite adaptor protein ASC (inflammasome adaptor protein apoptosis-associated speck-like protein containing CARD), and Caspase1 [87]. Several inflammasomes have been described to date, involving NLRP1, NLRP2, NLRP3, NLRC4 (also known as IPAF), NLRP6, and the DNA-sensing AIM2 (absent in melanoma-2) and RIG1 (retinoic acid-inducible gene-1) receptors. NLRP3 in particular can sense a wide range of PAMPs and hence is of particular importance in the development of acute and chronic inflammatory responses. These inflammasomes cleave pro-Caspase1 to active Caspase1, which is regarded as a key mediator of inflammatory processes. Caspase1 in turn cleaves pro-IL-1β and pro-IL-18 into their active forms. In addition to processing IL-1, Caspase1 executes a quick and programmed cell death response termed pyroptosis. Pyroptosis is a Caspase1-dependent inflammatory process of cell self-destruction to eliminate cellular niches that favor microbial growth [88]. Other caspases like Caspase3 and Caspase4 have also been implicated as playing a role in regulating mucosal recovery and tissue healing postinfection in OM [49].

We, and others, have previously demonstrated that IL-1 is important for the recruitment of neutrophils to the ME [89, 90]. Additional studies have also shown that IL-1β, IL-2, IL-6, IL-8, TNF-α, and several other cytokines and chemokines are stringently regulated during OM and are present in the ME effusions [36•, 48, 79, 91, 92, 93••]. Moreover, mice deficient in the key inflammasome component ASC, which modulates Caspase1 recruitment and activation, were more susceptible to ME infection than wild-type mice [47•]. This heightened susceptibility was associated with decreased activation of IL-1β considered due to reduction in active Caspase1, which, as noted above, is regarded as a key mediator of inflammatory processes and pyroptosis [88]. These data indicate that the inflammasome and the NLRPs play a role in the recovery from OM.

DNA Receptors

PRRs that can detect pathogenic DNA/RNA include the RLRs, AIM2, and TLR9. In addition, Pol-III can transcribe

bacterial DNA into RNA, where it can be sensed by RIG1 or MDA5 (Melanoma Differentiation-Associated protein 5) receptors which function as cytosolic DNA/RNA sensors alerting innate immunity to viral and bacterial RNA. These receptors act via proteins associated with the mitochondria (i.e., IPS1; interferon-β promoter stimulator 1) and the endoplasmic reticulum (ER) to induce IRF3 and IRF7 activation, leading to IFN production and thereby inducing inflammation. Hence, the RLRs cross talk with TLRs signaling through MyD88-dependent and IPS1-dependent pathways [57]. On the other hand, AIM2 receptors directly sense bacterial DNA in the cytosol and complex with the adaptor molecule ASC and pro-Caspase1 to form an inflammasome complex, leading to Caspase1 activation and subsequent cleavage and activation of the pro-inflammatory cytokines IL-1β and IL-18 [94]. In OM animal models, the genes encoding many of the DNA sensing molecules are significantly regulated during OM [48]. Experimentally, RIG1 gene expression was upregulated shortly after NTHi-induced infection, similar with AIM2 [51]; meanwhile, mice deficient in TLR9 exhibit prolonged OM phenotype [47•]. In otitis-prone patients, the expression levels of TLR9 and RIG1 mRNA has been found to be lower than in normal patients [61], possibly linking them to OM reoccurrence.

Additional PRRs

Besides TLRs, NLRs, and RLRs, innate immune responses can also be triggered by the detection of residual DNA or carbohydrate molecules from invading pathogens or damaged/dying cells. Those receptors include the members of the PGRP (peptidoglycan recognition proteins) and CLRs. PGRPs act both as sensors and scavengers of peptidoglycan and modulate the level of the host immune response to the presence of infectious agents [95]. The CLRs comprise a large family of receptors that recognize carbohydrate residues. They are divided into 17 groups based on their structural features and homology [96]. The roles of PGRPs and CLRs in the pathogenesis of OM have only begun to be elucidated. Recent clinical data from patients with OME and chronic OM showed increased mRNA expression of CLRs and CLR-related molecules such as *Dectin-1*, *MR1*, *MR2*, *Mincle*, *Syk*, *Card9*, *Bcl10*, *Malt1*, *Src*, *Dec205*, *galectin1*, *Tim3*, *Trem1*, and *DAP12* in ME effusions [97]. However, further functional studies assessing the role of these genes in OM development and resolution are needed to determine their functional role in OM pathogenesis and recovery.

Conclusions

The results of the studies reviewed above indicate that innate immunity plays a central role in the pathobiology

and resolution of AOM. Deficiencies in any of the innate immune receptors and signaling molecules that have been examined to date in animals have resulted in the persistence of bacterial OM beyond the period normally observed in wild-type animals. Moreover, association studies in human patients have linked polymorphisms in innate immune receptor, signaling, and effector genes to OM proneness. Thus, virtually, all of the innate immune pathways examined to date appear to be required for the appropriate resolution of OM. Some pathways do, however, appear to be more critical than others. A prominent example is the deletion of the TLR adaptor molecule MyD88 in mice results in the persistence of NTHi-induced OM for several weeks, as compared to a few days for wild-type mice [71]. In contrast, the deletion of the alternative TLR4 adaptor TRIF results in a much milder OM phenotype [50] likely due to the fact that MyD88 operates downstream from many more TLRs than does TRIF. Nonetheless, all deficiencies appear to decrease the host tolerance mechanisms and increase susceptibility to inflammation.

While many individual PRRs appear to be required for normal OM resolution, the results from animal studies also make it clear that there is significant collaboration, complementation, and some redundancy in innate sensing through the multiple PRRs present. For example, the deletion of the inflammasome adaptor ASC hindered the normal timely OM resolution in mouse models due to decreased IL-1β levels [47•]. However, all mice eventually cleared the ME infection. Such an outcome is to be expected since there are other PRR pathways which can redundantly, albeit less efficiently, activate many of the same effector genes as those whose expression is most effectively induced by the inflammasomes. However, it is apparent that these alternative pathways are not enough to mediate normal resolution on their own.

Innate immunity also involves contributions from phagocytic cells like neutrophils and macrophages capable of direct migration, microbial uptake, and clearance. Pro-inflammatory molecules like TNF-α, IL-1β, and CCL3 appear to play a key role in promoting cellular migration into the ME and/or activation of the cells for microbial clearance. The addition of recombinant TNF-α to the guinea pig ME was sufficient to induce an inflammatory response in the absence of microbial infection [90], while a knockout mouse OM model showed that the lack of TNF reduced both neutrophilic migration into the ME and macrophage phagocytosis. Interestingly, the addition of recombinant CCL3, a chemokine expressed at high levels during the course of OM, abolished infection by restoring normal macrophage phagocytic function in the knockout mouse. Similarly,

exogenous CCL3 aided the return of phagocytic function in TLR2-, MyD88- or TNF-deficient macrophages. Future novel therapeutic approaches to OM would focus on small molecules that can boost key innate immune mechanisms in addition to factors that block microbial virulence at the host level. For instance, the use of infliximab (monoclonal TNF-α antibody) reduced the inflammatory activity of OM in animal models [98•, 99]. It would be interesting to see if the use of antibodies like canakinumab (monoclonal anti-IL-1β antibody) would have similar effects. Similarly many TGFβ-targeting drugs have been developed and could be potential therapy targets. A recent study showed that the inhibition of TGFβ could alleviate secondary bacterial pneumonia infections in the lungs by impeding the cellular adhesins signaling following an influenza infection [100]. Naturally, the influenza viral infection activates TGFβ, which enhances bacterial adherence leading to increased host susceptibility to coinfections. It would be of value to evaluate the potentials of these drugs in OM.

In summary, studies performed to date indicate a critical role for many innate immune pathways in OM resolution. However, many PRRs and their downstream effectors have yet to be evaluated. Additional studies will be required to provide a more complete understanding of innate immune sensing and contributions to OM and hence improve therapeutic targets.

Abbreviations: *AIM2* interferon-inducible protein 2, *ASC* apoptosis-associated speck-like protein containing CARD, *CARD* caspase recruitment domain, *CpG DNA* DNA containing cytosine–guanine repeats linked by phosphodiester bonds, *dsRNA* double-stranded RNA, *ER* endoplasmic reticulum, *ERK* extracellular signal-regulated kinase, *IL-1* interleukin-1, *JNK* c-Jun amino-terminal kinase, *IRAK1* interleukin-1 receptor-associated kinase 1, *MAPK* mitogen-activated protein kinase, *NALP* NACHT leucine rich repeat (LRR) and pyrin domain (PYD) containing 3, *NFκB* nuclear factor κB, *p38* mitogen-activated protein kinase 1, *P50* subunit of NFκB that forms a heterodimer with P65, *P65* NFκB subunit, *RIG1* retinoic acid inducible gene protein 1, *TAK1* TGF-beta activated kinase 1, *TNF* tumor necrosis factor.

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