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The NLRP3 inflammasome as a target for sensorineural hearing loss

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ABSTRACT

Sensorineural hearing loss is the most common type of hearing loss in adults and occurs due to damage of the inner ear caused by a range of factors including ageing, excessive noise, toxins, and cancer. Auto-inflammatory disease is also a cause of hearing loss and there is evidence that inflammation could contribute to hearing loss in other conditions. Within the inner ear there are resident macrophage cells that respond to insults and whose activation correlates with damage. The NLRP3 inflammasome is a multi-molecular pro-inflammatory protein complex that forms in activated macrophages and may contribute to hearing loss. The aim of this article is to discuss the evidence for the NLRP3 inflammasome and associated cytokines as potential therapeutic targets for sensorineural hearing loss in conditions ranging from auto-inflammatory disease to tumour-induced hearing loss in vestibular schwannoma.

KEY WORDS

Sensorineural hearing loss, inflammation, cochlea, NLRP3 inflammasome, interleukin-1

INTRODUCTION

Hearing loss (HL) is the leading cause of years lived with disability in the U.K. (*1*). Approximately 12 million U.K. adults have HL, which is equivalent to one in five people (RNID prevalence estimates using Office for National Statistics population data, 2018), and is expected to rise to 14.2 million by 2035 because of the ageing population. HL leads to communication difficulties between family members, colleagues, and friends. HL is associated with poorer education and employment prospects, anxiety and depression, poor social interactions and isolation, an increased risk of dementia and reduced quality of life (*2*). Conductive hearing loss (CHL) occurs when sound is prevented from passing through the outer (pinna and ear canal) and middle (tympanic membrane and ossicles) ear (Figure 1A). Far more common in adults is sensorineural hearing loss (SNHL) where there is damage to the inner ear (cochlea and auditory nerve; Figure 1B). Important risk factors for SNHL, which is generally regarded as permanent, include age and excessive noise exposure. With a population prevalence greater than diabetes or cancer, it is disappointing that HL receives <1% of health research funding (*3*). There are currently no approved drugs for SNHL. Hearing aids, the most common intervention for HL, can improve the user's quality of life (*4*). Nonetheless, it is estimated that only 17% of people who could benefit from hearing aids use them (*5*).

Inflammation is emerging as a potentially common and causative factor for HL. During normal ageing, chronic inflammation occurs in both the peripheral and central auditory pathway (*6*). Cross-sectional data from the MRC National Study of Hearing shows a significant association between high white blood count and poorer hearing, and this association strengthens with age (*7*). There is extensive evidence that pro-inflammatory cytokines are induced in the cochlea after noise exposure (*8*). Therefore, cochlear damage and HL associated with excessive noise exposure may be linked to inflammatory processes in the cochlea (*9*). In addition, some health treatments may result in an inflammatory response and HL. For example, inflammation-induced damage to the inner ear has been implicated in HL

after radiotherapy treatment for head and neck cancer (*10*). Also, deterioration in performance of cochlear implants, a neural prothesis implanted in the cochlea of individuals with profound HL, is associated with inflammation at the cochlear tissue-electrode interface (*11*). The aim of this article is to discuss how inflammation contributes to SNHL, and how the NLRP3 inflammasome and associated cytokines represent therapeutic targets for SNHL ranging from, auto-inflammatory disease, to tumour-induced HL in vestibular schwannoma.

INFLAMMATION AND THE NLRP3 INFLAMMASOME

Inflammation is an immune system response and is vital for host protection against infection and injury (*12*). However, inflammation is also known to be destructive causing substantial tissue damage and contributes to the progression of many diseases (*13*). Cells of the innate immune system such as macrophages perceive pathogen and endogenous danger signals through pattern recognition receptors, which leads to production of pro-inflammatory cytokines. At the heart of many damaging inflammatory processes is a complex called the NLRP3 inflammasome (*14*). The NLRP3 inflammasome complex at its most basic level is composed of a cytosolic pattern recognition receptor called NLR family pyrin domain containing 3 (NLRP3), an adaptor protein called apoptosis-associated speck-like protein containing a CARD (ASC), and a protease called caspase-1 (Figure 1C). The cochlea contains a resident population of macrophages that respond to damage by increasing the expression of pro-inflammatory molecules and by increasing in number (*15*). Macrophage NLRP3 senses a diverse range of danger signals and through a complex and partially defined activation pathway involving post-translational modifications, and organelle stress. Activated NLRP3 nucleates the oligomerisation of ASC into a large platform (or speck) upon which caspase-1 is activated (*16*). This complex is called the NLRP3 inflammasome. Active caspase-1 processes inactive cytokine precursors pro-IL-1β and pro-IL-18 into their pro-inflammatory secreted forms. Caspase-1 also cleaves gasdermin D enabling its N-terminal fragment to oligomerise and form pores in the plasma membrane, providing a conduit for IL-1β and IL-18

secretion, and leading to a pro-inflammatory form of cell death called pyroptosis (*17*). Once released IL-1β and IL-18 bind to their cognate receptors on target cells (IL-1R1 and IL-18Rα/β respectively) and elicit downstream inflammatory responses including the production of other inflammatory mediators such as IL-6. Given NLRP3's role in disease processes there is great interest in the clinical development of NLRP3 inhibitors (*18*), and inhibitors of IL-1 signalling are already used in some clinical settings (*19*).

A spectrum of autoinflammatory diseases arise from activating mutations in the *NLRP3* gene and are called the cryopyrin-associated periodic syndromes (CAPS). The CAPS vary and overlap in severity from mild to severe with familial cold autoinflammatory syndrome (FCAS) representing the mild phenotype, Muckle–Wells syndrome (MWS) representing the moderate phenotype, and neonatal-onset multisystem inflammatory disease (NOMID)/chronic infantile neurologic cutaneous articular syndrome (CINCA) representing the severe phenotype (*20*). The CAPS syndromes are multi-system and cause inflammatory flares, urticaria, fever, fatigue, SNHL, conjunctivitis and other ocular manifestations, and central nervous system manifestations ranging from headache to more severe conditions (*20*). In a prospective clinical study of 57 patients with CAPS, SNHL was greatest in patients with NOMID or in patients where the phenotype was overlapping between NOMID and MWS, and correlated with increased cochlear inflammation as determined by fluid-attenuated inversion recovery (FLAIR)-MRI (*21*). Recently a non-syndromic SNHL caused by a missense mutation in *NLRP3* (Deafness, autosomal, dominant 34 (DFNA34)) was reported and suggested to depend upon local NLRP3-dependent inflammation from cochlear macrophages (*22*). Licenced therapies for CAPS include canakinumab - a humanised anti-IL-1β monoclonal antibody, anakinra – a recombinant form of the naturally occurring IL-1 receptor antagonist (IL-1Ra), and rilonacept – a fusion protein containing the IL-1 binding portion of IL-1R1 (*23*). First line treatment is canakinumab given once every two months at a dose of 150 mg subcutaneously. Second line of treatment is anakinra given at 100 mg subcutaneously daily (*23*). In patients with DFNA34 treated with anakinra at 200 or 100 mg daily for five months the SNHL had either completely

or markedly resolved (*22*). Hearing is generally stabilised in people with CAPS treated with anti-IL-1 therapy, with better treatment outcomes in younger people (*24*). Further, from a study of CAPS and DFNA34 families, a hearing threshold level poorer than 60 dB and cochlear enhancement on FLAIR-MRI are associated with a poor audiologic outcome in response to anakinra (*25*). A case study of a three-year-old boy with suspected and then confirmed MWS showed a complete resolution of SNHL upon treatment with anakinra (*26*). These studies suggest it can be possible to identify optimal therapeutic windows in CAPS patients for the recovery of SNHL in response to anti-IL-1 therapies.

Pre-clinical validation for NLRP3-dependent inflammation in the cochlea causing deafness comes from several recent studies. A transgenic knock-in mouse expressing the NOMID D301N mutation in NLRP3 was crossed with $Gfi1^{Cre}$ (growth factor independent 1 transcriptional repressor) knock-in mice to cause inner ear hair cell-specific expression of $NLRP3^{D301N}$. These $Nlrp3^{D301NneoR/+}$; $Gfi1^{Cre/+}$ mice had severe hearing loss (*27*). Another study in mice using conditional CX3CR1-specific expression of D301N NLRP3 found that in response to intraperitoneal lipopolysaccharide injection the mutant mice developed hearing loss, and that this was attenuated with IL-1Ra and the NLRP3 inhibitor MCC950 (*28*). In summary, the CAPS literature suggests that cochlear inflammation driven by NLRP3 promotes SNHL and is sensitive to anti-IL-1 therapies.

SNHL AND INFLAMMATION IN NON-CAPS CONDITIONS

Meniere disease (MD) is an inner ear disorder arising from different disease aetiologies and results in fluctuating low-frequency SNHL with tinnitus in the affected ear and vertigo as its core symptoms (*29, 30*). The prevalence of MD varies depending upon ethnic background and in Japan has a prevalence of 3.5 per 100,000, while in Finland the prevalence is 513 per 100,000 (*30*). The prevalence of autoimmune disease is greater in people with MD than in the general population (*31*), and autoimmune mechanisms are suggested to be responsible for 6

and 16% of unilateral and bilateral forms of MD, respectively (*32*). Peripheral blood mononuclear cells (PBMCs) isolated from people with MD have higher levels of IL-1β than healthy controls (*33*). Autoimmune inner ear disease (AIED) appears clinically similar to MD but is distinguished by the occasional presence of vertigo during attacks of HL compared to MD where vertigo is present with every attack (*34*). Up to 70% of patients with AIED respond to corticosteroid treatment (*35*), and in corticosteroid non-responders there are elevated plasma levels of IL-1β (*36*). In a phase I/II open-label, single-arm clinical trial of anakinra in corticosteroid-resistant AIED patients, daily doses of 100 mg anakinra delivered by subcutaneous injection for 84 days resulted in an improvement in hearing in a cohort of the patients (*37*). Interestingly a caspase-7-dependent 28 kDa pro-inflammatory form of IL-1β is produced in PBMCs from people with AIED (*38*).

Sudden sensorineural hearing loss (SSNHL) is a rapid decline in inner ear hearing, instantly or over a few days, and usually in one ear. SSNHL affects 5-30 cases per 100,000 per year (*39*) and is one of the most common emergencies in Ear, Nose and Throat practice. It results in difficulty hearing in background noise, reduces sound localisation, is sometimes accompanied by tinnitus, and reduces quality of life (*40*). Investigations rarely reveal the cause so most cases are termed idiopathic. Steroids to reduce the supposed inflammatory response to hyperbaric oxygenation comprise the mainstay of treatment for idiopathic SSNHL. However, there is a lack of evidence on duration and delivery route (oral or intratympanic delivery; (*41*)). Trials with no-treatment or placebo arms are scarce but spontaneous recovery of ISSNHL may be close to 60% (*42*).

A number of other studies in rare conditions highlight a role for inflammation in hearing loss. SLC29A3 spectrum disorder is a rare multi-system autoinflammatory syndrome presenting with a spectrum of severity including HL. A two-year-old boy with SLC29A3 spectrum disorder and SNHL was treated with the anti-IL-6 drug tocilizumab for 18 months, after which his SNHL had improved and stabilised (*43*). A patient with another rare auto-immune disease, Cogan's syndrome, also responded to tocilizumab with recovery and

stabilisation of SNHL (*44*). Treatment of an 18-year-old with SNHL, diagnosed with systemic lupus erythematosus with macrophage activation syndrome, using intravenous methylprednisolone caused a near complete resolution of his SNHL (*45*). In genetic mouse models of deafness, dexamethasone protected hearing by preventing hair cell loss in the cochlea and reduced the numbers of cochlear macrophages (*46*). A network analysis of the metabolome of chemical drugs reported to affect SNHL identified drugs that are most likely to affect different types of SNHL, one of which was dexamethasone (*47*).

The term inflammaging describes the appearance of chronic low-grade inflammation that is associated with the aging process, functional decline, and age-related disease (*48*). Experimental evidence in mice suggests that the NLRP3 inflammasome controls the age-related inflammation leading to functional and cognitive decline (*49*). Age-related hearing loss (ARHL) is a common chronic sensory deficit experienced by the elderly, and is associated with significant psychological and medical morbidity, including cognitive decline (*50*). Indeed, ARHL is suggested to be a promising modifiable risk factor of dementia (*51*). ARHL may occur as a combination of CHL arising from defects with the middle ear, and SNHL (*52*). Within cohorts of elderly subjects elevated levels of inflammatory markers such as IL-6 and C-reactive protein are associated with increased HL (*53, 54*), suggesting ARHL could have an inflammatory component. Analysis of the cochleae of aged mice with ARHL identified a number of differentially regulated genes relative to young control mice, which include inflammatory signalling molecules such as NF-κB, IL-1β, and caspase-1, amongst others. Further, immunohistochemistry of cochlear sections from aged mice identified IL-1β localisation to elements of the cochlea including the organ of Corti (*55*), suggesting that the inflammation contributing to ARHL could be localised in the cochlea. Noise-induced HL may also involve NLRP3-dependent inflammation. A study using miniature pigs discovered that acoustic trauma caused increased NLRP3, IL-1β, IL-18 and active caspase-1 in the cochlea (*56*). Similar results were observed in a mouse model of noise-induced HL with increased inflammasome activation in mouse cochlea exposed to excessive noise. In this study anakinra,

and a NLRP3 inflammasome inhibitor, oridonin, were shown to alleviate noise-induced HL (*57*). Ototoxic drugs such as aminoglycoside antibiotics also cause SNHL in children and adults. In a mouse model of drug-induced SNHL caused by injection of the antibiotic kanamycin and furosemide, inflammasome activation was detected in the cochlea coinciding with hair cell loss and this was prevented by treatment with the NLRP3 inhibitor oridonin (*58*). The effects of cisplatin on the cochlea, another ototoxic drug, are also suggested to depend upon the NLRP3 inflammasome (*59*).

SNHL can also be caused by vestibular schwannomas (VS), benign tumours arising from the Schwann cells lining the vestibular nerves. Most commonly VS are unilateral and are sporadic, although in patients with the tumour predisposition syndrome *NF2*-schwannomatosis (*NF2*-SWN), the tumours occur bilaterally. *NF2*-SWN patients describe HL as their greatest problem (*60*). Preserving hearing in patients with *NF2*-SWN while managing VS is complex, as preserving hearing is not the only outcome being considered during treatment (*61*). There are studies suggesting that the SNHL due to VS is not solely accounted for by compression of the nerve by the growing tumour. A retrospective analysis of 75 patients with VS found a statistically significant association with tumour diameter and severity of low frequency SNHL, but not to the severity of mid or high frequency SNHL (*62*). Another study reported that preoperative hearing levels were not related to tumour volume assessed by MRI (*63*), and another study looking at the course of hearing in a cohort of 156 patients with VS found that the HL at diagnosis was not related to tumour volume (*64*). A recently published comprehensive retrospective analysis of 477 patients with unilateral sporadic VS found no association between tumour size or position and SNHL, suggesting other aspects of the VS microenvironment affect hearing (*65*). It is possible that inflammation contributes to SNHL in VS. The VS microenvironment is highly inflammatory with high levels of immune cell recruitment (*66*). One study found increased levels of the macrophage marker CD163 in specimens taken from patients with poor hearing compared to good hearing and that this relationship was independent of tumour volume (*67*). In tumour secretions from biopsied VS,

significantly raised levels of Fibroblast Growth Factor 2 (FGF2) were associated with specimens from patients with good hearing alongside substantially elevated levels of the IL-1 signalling inhibitor IL-1Ra (*68*). In a mouse model of NF2 VS, treatment with losartan, an antihypertensive drug, prevented HL, and this correlated with reduced inflammation, including reduced levels of IL-6 and IL-1β (*69*). Pathway analysis of microarray data from 80 VS identified an upregulation of the NLRP3 pathway. The increase in NLRP3 was further validated in an additional 30 VS specimens and a significant increase in NLRP3 expression was found in VS samples from patients with poor hearing compared to those from patients with good hearing (*70*). In a retrospective study of 1698 patients with SNHL, 43 were found to have VS and 11 of them (34%) showed a good recovery of SNHL with prednisolone treatment, and this response was independent of tumour size (*71*). These studies suggest that inflammation could cause SNHL in VS.

CONCLUSIONS

There is evidence to suggest the NLRP3 inflammasome is a contributor to the inflammation that causes SNHL in a number of conditions. The reports showing that treatment of the CAPS with anti-IL-1 therapies can alleviate SNHL, and the identification of NLRP3 in SNHL caused by other conditions, such as VS, suggests that anti-IL-1 therapies could be repurposed to treat SNHL beyond the CAPS.

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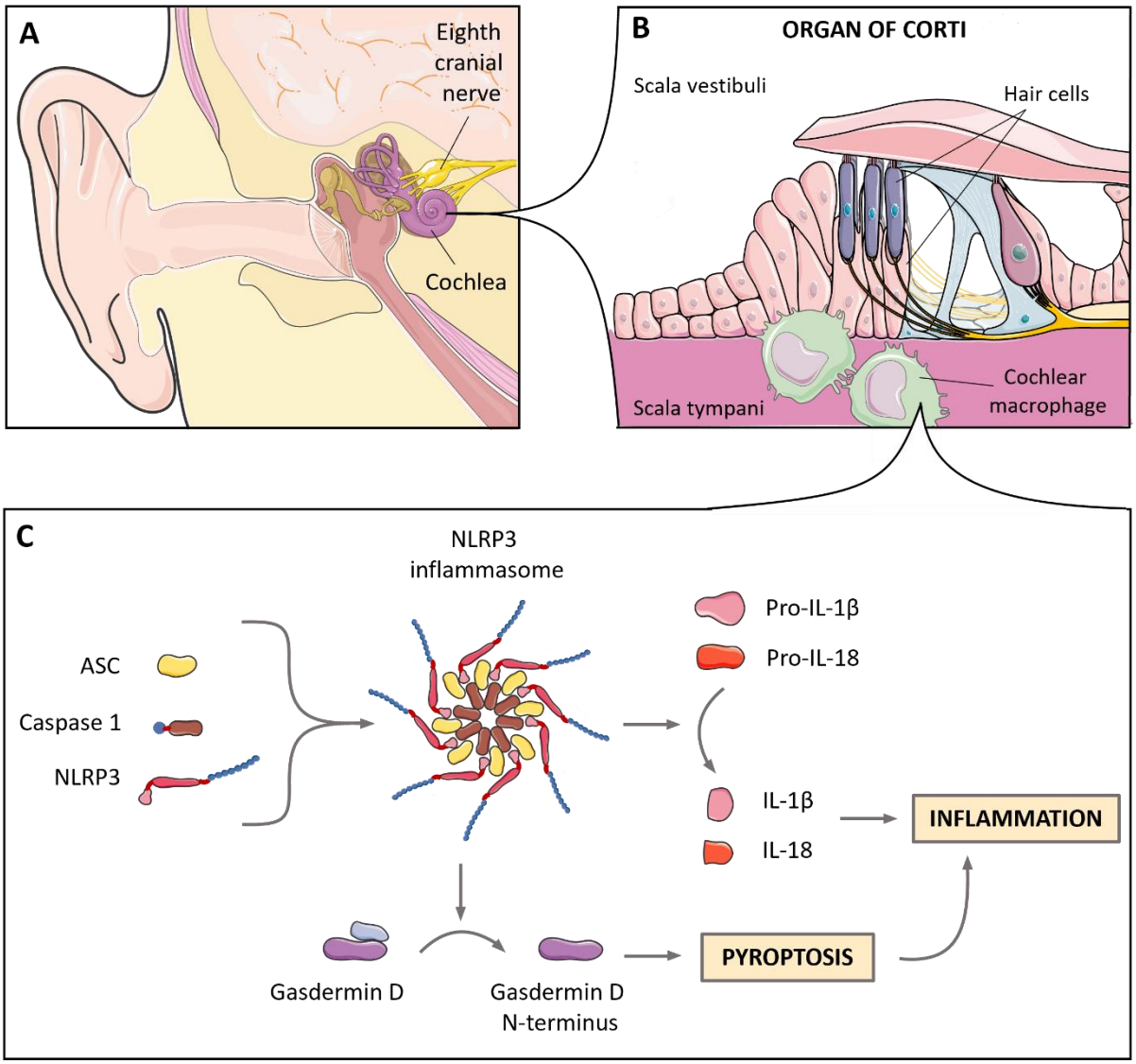

Figure 1. NLRP3 activation in the cochlear macrophages of the inner ear. (A) SNHL occurs in the inner ear where the eighth cranial nerve innervates the cochlea. (B) Within the cochlea, the inner and outer hair cells of the organ of Corti mediate the signal transduction between the cochlea and the eighth cranial nerve. Cochlear macrophages aid in the phagocytosis of cellular debris in healthy tissue but promote inflammatory signalling in cases of SNHL. (C) NLRP3 inflammasome activation occurs in cochlea macrophages leading to pro-inflammatory cytokine release and pyroptosis, a pro-inflammatory form of cell death. Abbreviations: NLR family pyrin domain containing 3 (NLRP3), sensorineural hearing loss (SNHL), apoptosis-associated speck-like protein containing a CARD (ASC), interleukin-1β (IL-1β), interleukin-18 (IL-18). Figure created with Servier Medical Art, licensed under a Creative Common Attribution 3.0 Unported License (http://smart.servier.com/).