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REVIEW ARTICLE

Macrophages in the cochlea; an immunological link between risk factors and progressive hearing loss

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Abstract

Macrophages are abundant in the cochlea; however, their role in hearing loss is not well understood. Insults to the cochlea, such as noise or insertion of a cochlear implant, cause an inflammatory response, which includes activation of tissue-resident macrophages. Activation is characterized by changes in macrophage morphology, mediator expression, and distribution. Evidence from other organs shows activated macrophages can become primed, whereby subsequent insults cause an elevated inflammatory response. Primed macrophages in brain pathologies respond to circulating inflammatory mediators by disproportionate synthesis of inflammatory mediators. This signaling occurs behind an intact blood–brain barrier, similar to the blood-labyrinth barrier in the cochlea. Local tissue damage can occur as the result of mediator release by activated macrophages. Damage is typically localized; however, if it is to structures with limited ability to repair, such as neurons or hair cells within the cochlea, it is feasible that this contributes to the progressive loss of function seen in hearing loss. We propose that macrophages in the cochlea link risk factors and hearing loss. Injury to the cochlea causes local macrophage activation that typically resolves. However, in susceptible individuals, some macrophages enter a primed state. Once primed, these macrophages can be further activated, as a consequence of circulating inflammatory molecules associated with common co-morbidities. Hypothetically, this would lead to further cochlear damage and loss of hearing. We review the evidence for the role of tissue-resident macrophages in the cochlea and propose that cochlear macrophages contribute to the trajectory of hearing loss and warrant further study.

KEYWORDS

cochlea, hearing loss, immune memory, immune response, inflammation, macrophages, priming

1 | MACROPHAGES IN THE AUDITORY SYSTEM

The identification of the blood-labyrinth barriers (BLB) (Juhn & Rybak, 1981) resulted in the cochlea being regarded as immune-privileged. However, the cochlea has an immune capacity. Immune cells and inflammation have roles in the physiology and pathophysiology of hearing and hearing loss (Fujioka et al., 2006; Keithley et al., 2008; Ma et al., 2000; Wang et al., 2003). Cochlear macrophages and perivascular macrophage-like melanocytes (PVM/Ms) are resident in the cochlea of adult humans (Liu et al., 2018; O'Malley et al., 2016) and mice (Okano et al., 2008). Evidence from studies in

rodents has identified recruitment and infiltration of monocytes from the circulation into the cochlea after acoustic trauma (Hirose et al., 2005; Tan et al., 2008; Tornabene et al., 2006), hair cell ablation (Kaur, Zamani, et al., 2015b), ototoxicity (Sato et al., 2010) and in response to cochlear implantation (Bas et al., 2015; Farhadi et al., 2013). The roles of these populations of cochlear macrophages are still to be fully determined.

Macrophages are pleiotropic immune cells with roles in homeostasis and inflammation (Davies & Taylor, 2015; Ginhoux & Guilliams, 2016; Hoeffel & Ginhoux, 2018). Macrophages detect, phagocytose, and clear cellular debris and pathogens from tissues. They initiate inflammation and the subsequent anti-inflammatory reparative response (or tissue repair) by producing pro- and anti-inflammatory cytokines and chemokines, which signal to surrounding cells. Macrophages can be divided into tissue-resident and infiltrated macrophages. Tissue-resident macrophages reside in tissue or organ systems (Ginhoux & Guilliams, 2016; Krenkel & Tacke, 2017; Mu et al., 2021; Wang et al., 2019b) and primarily carry out homeostatic roles under steady state (healthy) conditions. Monocytes are recruited from the bone-marrow (hematopoietic system) into tissues following an insult or pathology and differentiate into macrophages, so called infiltrated macrophages. The distinction between long-lived resident and tissue-infiltrated macrophages in the cochlea and the value in being able to identify these populations and their origins will be addressed in this review.

1.1 | Hearing and auditory system

The auditory system comprises a number of anatomical structures from the pinna (external ear) to the auditory centres of the central nervous system (CNS). Once sound waves are converted to mechanical energy by the tympanic membrane and middle ear, pressure changes are then converted to neural signals through the action of specialized sensory hair cells within the cochlea. Neural signals are transmitted along the auditory nerve to the brain. The organization of the auditory system and cellular basis of hearing has been reviewed extensively (Cunningham & Tucci, 2017; Raphael & Altschuler, 2003). An overview of the anatomical regions referred to in this review is illustrated in Figure 1. Hearing loss is a widespread problem with multiple causes, with almost 500 million people worldwide thought to have disabling hearing impairment (World Health Organisation, 2020). Understanding the role played by macrophages in driving the pathophysiology of progressive hearing loss is crucial in the development of future therapies. This is particularly timely given the absence of effective pharmacological treatments for the prevention or reversal of hearing loss.

Each stage in the auditory system can be damaged, with resulting impairment of hearing function. The earliest identified sites of injury following an insult are to the synapses between inner hair cells and spiral ganglion neurons (SGNs) (Fernandez et al., 2015; Kujawa & Liberman, 2009; Sergeyenko et al., 2013). Hair cells can become damaged and die following both acute and chronic insults (Bohne & Harding, 2000). In humans, hair cells are terminally differentiated and therefore cannot self-renew following significant damage (Mittal et al., 2017; Shu et al., 2019). Spiral ganglion neurons, which make synaptic connections between the hair cells and the neurons of the cochlear nucleus (Glueckert et al., 2005) (indicated in Figure 1c), can become damaged and degenerate. Damage or atrophy of the stria vascularis disrupts cochlear function (Neng et al., 2015; Zhang et al., 2013). Repeated exposure of the auditory system to insults will cause further damage to these structures, resulting in a gradual loss of function leading to progressive hearing loss. This review will discuss tissue-resident macrophages of the central (microglia) and peripheral (cochlear macrophages) auditory system and propose that tissue-resident cochlear macrophages contribute to the progression of hearing loss.

Cochlear macrophages have functional similarities with microglia in the central nervous system. Both cell types adopt characteristic morphologies and phenotypes, which are plastic and responsive to changes in the local tissue micro-environment. Macrophage morphology is an indicator of function and can identify a change in the state of immune activity (Li & Barres, 2018). The distribution, abundance, and morphology of cochlear macrophages across different regions of the adult cochlea under homeostatic conditions, and after different cochlear insults, have been investigated in both human and animals using mainly histological techniques. A summary of the distribution and morphological characteristics of macrophages in the structures of the auditory system is presented in Figure 1. Figure 2 details the cellular expression profiles of the macrophage populations. Compared to other tissue-resident populations of macrophages, the cellular expression profiles of cochlear macrophages and the markers which have and can be used to identify these cells is less well documented; this needs to be considered when drawing conclusions in the field. This review draws together the data and principal findings that describe the cochlear macrophage populations in steady state and after three common causes of damage or change to the cochlea, namely exposure to noise, age-associated changes, and cochlear implantation (which can be considered an unwanted side-effect of the intervention).

1.2 | Tissue-resident macrophage populations

Tissue-resident macrophage populations with distinct features have been identified and characterized in different tissues, including $CCR2^{-}$ cells in the heart and microglia in the eye and brain (Li & Barres, 2018; Wang et al., 2019b). These resident cells have roles in development, homeostasis, and pathologies including those associated with aging (Li & Barres, 2018; Wang et al., 2019b). Tissue-resident macrophages are distributed across the central (microglia and perivascular macrophages) and peripheral (cochlear macrophages and perivascular macrophage-like melanocyte (PVM/Ms)) auditory system. The central auditory system (part of the CNS) begins at the cochlear nucleus in the brainstem from where pathways project through to the auditory cortex (Figure 1d). Microglia and macrophages reside within the CNS. The methods, current limitations and value of being able to delineate between these cells is discussed below. The peripheral auditory system begins at the proximal auditory nerve leading into the cochlea

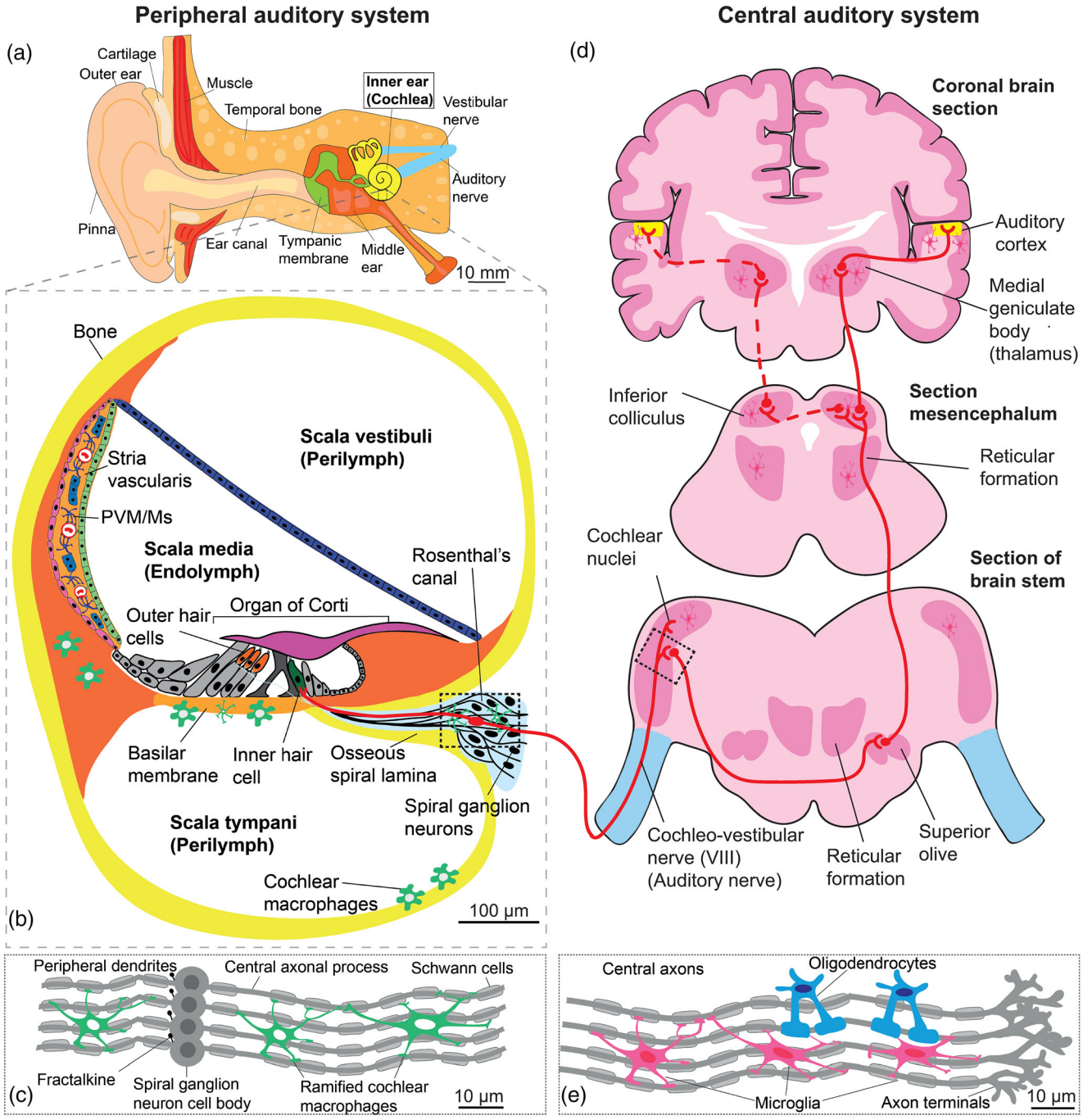
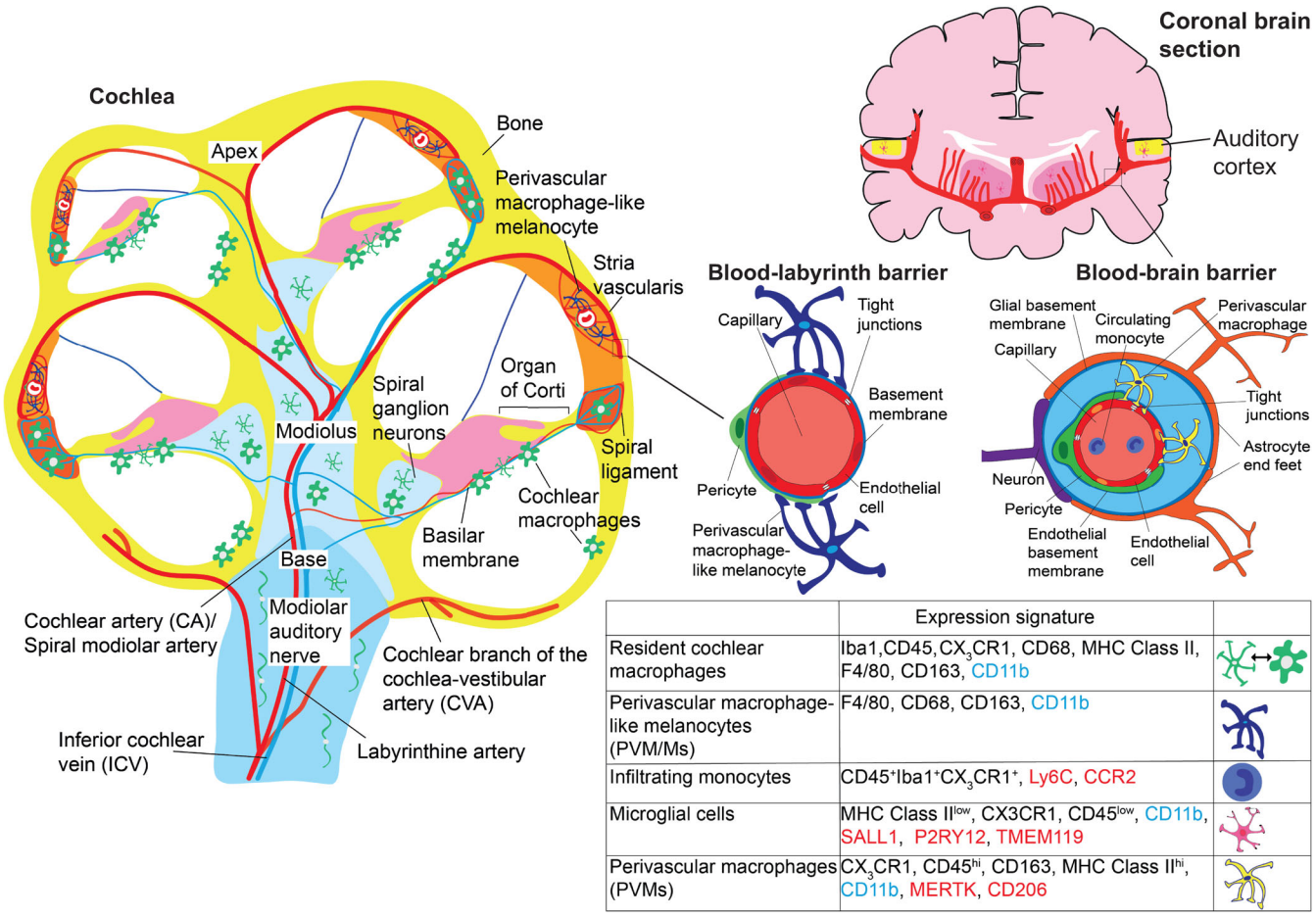


FIGURE 1 Overview of the organization and anatomy of the auditory system, highlighting the distribution of macrophages. The auditory system comprises the peripheral and central regions (left and right columns). The peripheral auditory system (a) consists of the outer, middle, and inner part of the ear or cochlea. Hearing function relies on specialized soft-tissues, cartilage, and bone and associated vascular beds. A high magnification (b) cross-section of a cochlear turn to identify the organ of corti, stria vascularis and Rosenthal's canal. The organ of corti contains the inner and outer hair cells, that detect and amplify sounds in a frequency-dependent manner. The stria vascularis regulates the ionic composition of the cochlear fluids and separates the internal cochlear environment from the systemic circulation. The modiolus, or bony central axis of the cochlea, contains Rosenthal's canal which houses the cell bodies of the spiral ganglion neurons (SGNs) which relay auditory information between the cochlea and central nervous system. An illustration (c) of the cellular organization of a region of the cochlear auditory nerve showing SGNs, which have peripheral and central axonal processes. Peripheral dendrites synapse with the hair cells. Cochlear macrophages have ramified processes that contact adjacent neurons. Centrally (d) the pathways project from the cochlear nucleus in the brainstem to the auditory cortex. The ascending primary auditory pathway relays signals from the cochlea along axons of spiral ganglion neurons to the cochlear nucleus in the brain stem. The auditory fibers cross the midline and relay to the superior olivary complex and inferior colliculus. The final relay is the medial geniculate body in the thalamus before the signal is relayed to the auditory cortex. An illustration (e) of the cellular organization of a region of the distal part of the auditory nerve, with centrally projecting axons of SGNs with axon terminals that synapse in the cochlear nuclei. Function within the auditory pathway relies on resident glial cells including non-myelinating and myelinating Schwann cells, oligodendrocytes and specialized sub-populations of macrophages



	Expression signature	
Resident cochlear macrophages	Iba1,CD45,CX_3CR1, CD68, MHC Class II, F4/80, CD163, CD11b	
Perivascular macrophage-like melanocytes (PVM/Ms)	F4/80, CD68, CD163, CD11b	
Infiltrating monocytes	$CD45^+Iba1^+CX_3CR1^+$, Ly6C, CCR2	
Microglial cells	MHC Class II^{low}, CX3CR1, $CD45^{low}$, CD11b, SALL1, P2RY12, TMEM119	
Perivascular macrophages (PVMs)	CX_3CR1, $CD45^{hi}$, CD163, MHC Class II^{hi}, CD11b, MERTK, CD206	

FIGURE 2 The distribution and expression signature of macrophage sub-populations in the human and murine auditory systems. Cochlear macrophages and microglia are located in different sub-structures and microenvironments across the auditory system (left panel). Perivascular macrophages are integral to the regulation of the permeability of the blood–brain barrier (BBB) and cochlear blood-labyrinth barrier (BLB) (right panel). The origins of the macrophages within sub-structures are reflected in the array of cellular markers which can be used experimentally to identify these cells. Several markers that identify different CNS glial populations have been identified; however, this selectivity has not been defined for macrophages in the peripheral auditory system. Greater delineation of the populations is needed to improve our understanding of the role of these cells in auditory homeostasis and hearing loss. Cell markers used to identify the populations vary in specificity from CD45, a pan leukocyte marker, to the commonly used microglia and macrophage marker Iba1. CD11b (highlighted in blue text) is expressed by all the resident macrophage populations described to date. Markers unique to sub-populations (red text) that could be used to differentiate between macrophage populations. Despite the progress, a definitive list of specific, reliable and experimentally tractable markers for cochlear macrophages is still needed and would be highly beneficial for advancing the field. See Table S1 for the citations associated with each cell marker

(Figure 1b). Macrophages reside within the peripheral auditory system.

Microglial precursors (Figure 2) infiltrate the brain early in the developing embryo. They arise from embryonic yolk sac precursors (Ginhoux et al., 2010) and maintain their population by self-renewal (Ajami et al., 2007; Lawson et al., 1992). The ability to identify the origin and kinetics of these cells in homeostatic or pathological tissue has been important in understanding their responses to other signaling cascades. Perivascular macrophages (PVMs), a second resident population in the brain, are located between the systemic circulation and CNS parenchyma at the blood–brain barrier (Galea et al., 2008; Galea & Perry, 2018; Lapenna et al., 2018; Varatharaj & Galea, 2017). PVMs are yolk sac-derived and are seeded in the brain early in development (Faraco et al., 2017; Goldmann et al., 2016; Lapenna et al., 2018). PVMs and microglia have roles in homeostasis and pathology. Importantly both brain resident populations, microglia and perivascular macrophages, are long-lived and sensitive to epigenetic modification (Haley et al., 2018; Keren-Shaul et al., 2017). This gives them the ability to develop a memory of previous insults to the tissue they reside in. It is this characteristic that may give tissue-resident macrophages a pivotal role in some pathologies.

1.3 | Tissue-resident cochlear macrophages

Cochlear macrophages become activated and change their morphology, abundance, and distribution in response to insults (Hirose et al., 2005; Lang et al., 2006; Liu et al., 2018; Noonan et al., 2020;

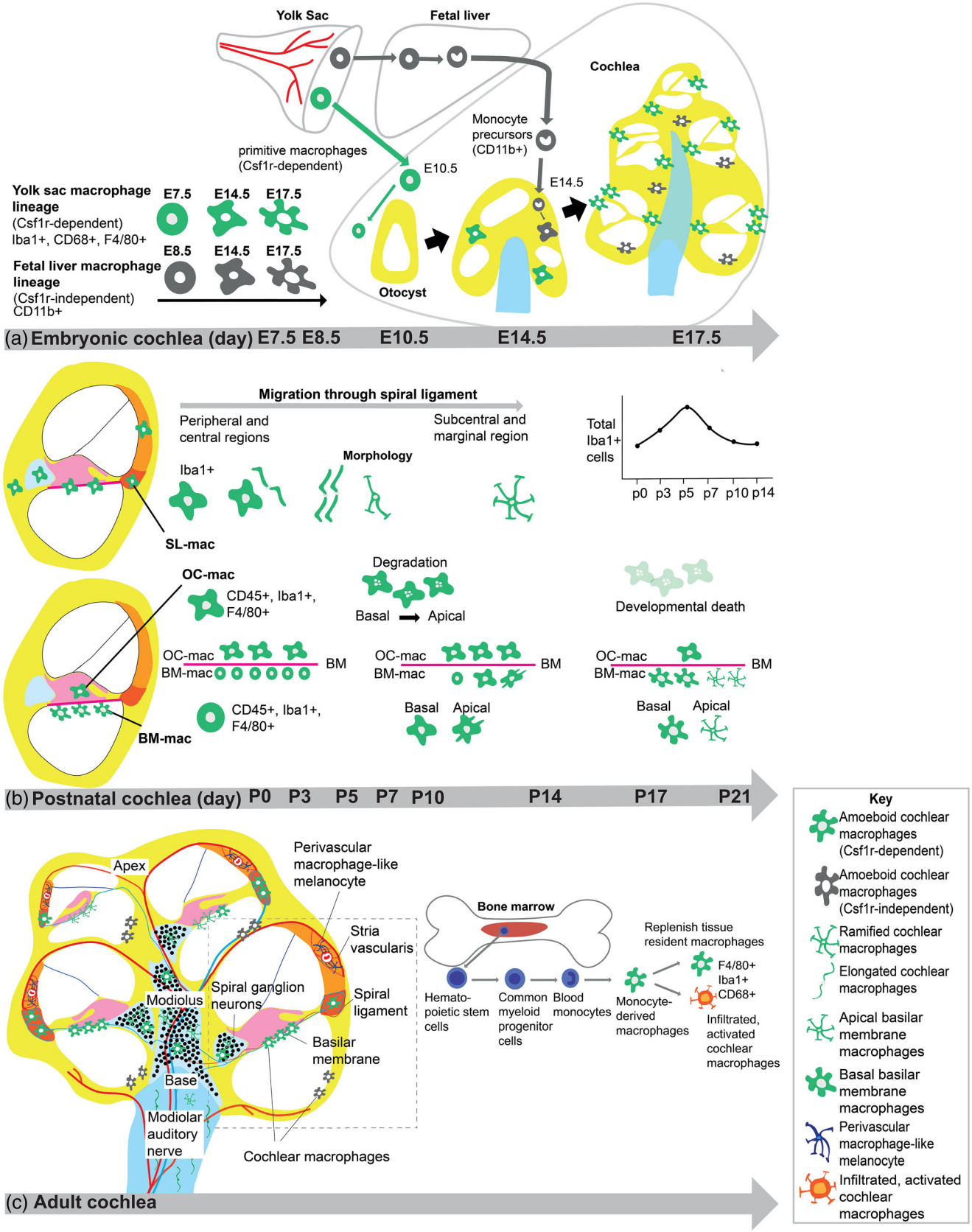


FIGURE 3 Legend on next page.

O'Malley et al., 2016; Okano et al., 2008; Okayasu et al., 2020). It is unclear whether and when these changes are protective or detrimental to cochlear function and this raises several questions, including: what are the roles of cochlear macrophages in injury/repair responses? What are the molecular mediators that control macrophage recruitment, signaling, and turnover and do these contribute to hearing loss? Can macrophages in the cochlea enter a state of altered activation referred to as primed, leaving them susceptible to disproportionate responses to secondary inflammatory insults? In order to improve hearing outcomes for people with hearing loss, we need to identify and understand how best to modulate the auditory system at a cellular level. This requires a major advance in our understanding of the biology of macrophages in the auditory system.

1.4 | Origin of macrophages in the cochlea

The dynamics of macrophage populations in the developing cochlea has been characterized most extensively in the mouse and are summarized in Figure 3 (Chen et al., 2018; Dong et al., 2018; Kishimoto et al., 2019; Okano et al., 2008). Colony stimulating factor 1 (Csf1) signaling, which is integral to the development and maintenance of microglia (Ryan et al., 2001), also controls seeding of the larger population of macrophages in the cochlea during development. A second population derived from the fetal liver, which is csf1r-independent (Kishimoto et al., 2019), is found in the modiolus and intraluminal surface of the perilymphatic space in the embryonic cochlea. Cochlear macrophages and perivascular macrophage-like melanocytes (PVM/Ms), (Ginhoux & Guilliams, 2016; Kishimoto et al., 2019; Okano et al., 2008), are resident in the adult cochlea. Cochlear macrophages persist from early post-natal stages (Dong et al., 2018) and they renew or maintain their population through infiltration of circulating monocytes (Hirose et al., 2005; Lang et al., 2006; Okano et al., 2008; Sato et al., 2010; Shi, 2010; Tan et al., 2008). Whether self-renewal also occurs through proliferation of the resident population, as is seen in the brain (Askew et al., 2017), is not clear. Perivascular macrophage-like melanocytes are found adjacent to blood vessels in the cochlea (Shi, 2010; Zhang et al., 2012). They have a turnover time of around 10 months in mice and are maintained through migration of monocytes into the cochlea (Shi, 2010). PVM/Ms contribute to the maintenance of the endocochlear potential (Zhang et al., 2013; Zhang et al., 2012) and the essential role of these cells in the cochlea is seen in a study where depletion of PVM/Ms in mice causes a reduction in endocochlear potential and hearing loss (Zhang et al., 2012).

2 | DISTRIBUTION AND MORPHOLOGY OF COCHLEAR MACROPHAGES UNDER HOMEOSTATIC CONDITIONS

2.1 | Organ of Corti macrophages

There is conflicting evidence regarding the existence of macrophages in the organ of Corti under homeostatic conditions. This uncertainty is likely due to variability in age at sample collection as much as the species studied and the methods applied to identify these cells. The organ of Corti in the mouse has been reported not to contain macrophages (Hirose et al., 2005; Yang et al., 2015). By contrast, analysis of human temporal bones identified Iba1+ macrophages with differing morphologies in the organ of Corti (Liu et al., 2018, 2019; O'Malley et al., 2016). Super-resolution structured illumination microscopy (SR-SIM) and transmission electron microscopy were used in the analysis of human tissue (Liu et al., 2018, 2019), these techniques have not been reported in the mouse. Macrophages in the organ of Corti are positioned along the perilymphatic side of the basilar membrane at the basal region of the tunnel of Corti and could be described as basilar membrane macrophages (Liu et al., 2018, 2019; O'Malley et al., 2016). In human tissue, these macrophages have been shown to have some cellular processes which penetrate through the basilar membrane (Okayasu et al., 2020) and which could engage in surveillance of synapses, a role demonstrated in brain microglia, at the hair cell: spiral ganglion neuron interface, though this has not been described.

2.2 | Basilar membrane macrophages

Basilar membrane macrophages show morphological differences between the apical and basal regions of the cochlea. Macrophages in the apical region of the sensory epithelium have a ramified morphology with long processes and a small cell body, in contrast amoeboid cells are reported in the basal turn (Frye et al., 2017, 2018; Yang et al., 2015). It is unclear whether this reflects a morphological difference associated with cells that are in steady-state and is due to location, or a difference due to a local change in the tissue associated with

FIGURE 3 Origins and maintenance of macrophages in the cochlea, through development. (a) Csf1r-dependent and csf1r-independent resident macrophages are found in the cochleae of the developing embryo. These subtypes vary in their origin, the markers they express, their abundance and distribution. Figure adapted from (Kishimoto et al., 2019). (b) The distribution and morphology of macrophages changes during postnatal development of the cochlea, this is most marked in the spiral ligament (SL) (Chen et al., 2018) organ of Corti (OC) and basilar membrane (BM) macrophages (Dong et al., 2018). (c) The distribution, morphology, and abundance of macrophages vary depending on the specific site within the tissue and these factors determine the function of the cells under homeostatic conditions and following a cochlear insult. The resident macrophage population is replenished by bone-marrow derived cells. See Figure 2 for a description of each cell type and the markers they express. Whether maintenance or replenishment of any of the resident macrophage populations in the adult cochlea occurs through self-renewal is not yet described in the literature

being in the low or high-frequency regions of the cochlea. High frequency regions of the cochlea appear more vulnerable to age-related changes. The hearing status is not reported for all the studies and would enable a closer correlation between intact sensory function, so a homeostatic state, and macrophage characteristics.

2.3 | Perivascular resident macrophage-like melanocytes

Perivascular resident macrophage-like melanocytes (PVM/Ms) are associated with blood vessels in the healthy adult stria (Neng et al., 2015). These cells have a branched morphology and ensheath the vessel walls with endfeet-like processes (Zhang et al., 2013; Zhang et al., 2012). Low numbers of cochlear macrophages have been reported in the spiral ligament, often around blood vessels (Liu et al., 2018) and amongst the fibrocytes (O'Malley et al., 2016).

2.4 | Spiral ganglion neuron macrophages

A morphologically distinct population of cochlear macrophages is distributed amongst the cell bodies of the spiral ganglion neurons. In the cases reported from a rat and human study, the macrophages are characteristic of homeostatic microglia (Fuentes-Santamaria et al., 2017; Liu et al., 2018). The rat and human modiolar auditory nerve is also populated with elongated macrophages (Figure 1c, 3c) with filamentous processes that contact adjacent neurons (Fuentes-Santamaria et al., 2017; Liu et al., 2019).

2.5 | Characterizing the distinct populations of macrophages in the auditory system

Resident and infiltrated macrophages produce pro-inflammatory and anti-inflammatory cytokines and chemokines following an immune event including IL-1β, TNF-α, TGF-β, CX3CR1, and IL-10. Drawing on the functional similarities between CNS microglia and cochlear macrophages is useful, as it highlights their complex and pivotal functions in tissue homeostasis and in diseased or injured states. Despite the advances, the characterization and identification of the distinct macrophage populations in the cochlea is limited by the relatively small number of published studies and methods used to characterize these cells. Compared to other tissue-resident populations of macrophages, the expression signature of cochlear macrophages and the markers, which can be used to identify these cells is less well documented. The detailed expression profiles of microglia and macrophages of the brain have been characterized using comparative transcriptomic studies (Crotti & Ransohoff, 2016; Li & Barres, 2018). Recent single-cell RNA sequencing of cochlear tissue after noise damage (Rai et al., 2020) and transcriptomic analysis of the C57 mouse cochlea has identified genes associated with macrophage activation (Su et al., 2020). Extending this work to identify and establish the set of markers expressed by cochlear macrophages using multiplex and comparative transcriptomic studies will enable more reliable identification of sub-populations and roles and comparison with other organ systems. To better understand the role of these cells, approaches are needed that enable source and region (cochlear sub-structure) specific macrophage information to be captured. This requires the site of isolated cells to be identified, or the signal to be detected in-situ in the tissue. In the brain microglia and infiltrated monocytes have been shown to display functional differences and contribute to disease pathologies differently, such as after ischemic stroke or in multiple sclerosis (Ritzel et al., 2015; Yamasaki et al., 2014). Markers such as TMEM119, which appears to be specific for microglia in both human and mouse, can be used to distinguish resident microglia from blood-derived macrophages (Bennett et al., 2016; Satoh et al., 2016). Being able to distinguish resident and infiltrated macrophages in the cochlea will help elucidate the precise function of each sub-population.

3 | DISTRIBUTION AND MORPHOLOGY OF COCHLEAR MACROPHAGES AFTER INJURY

Experimental models, predominantly in the mouse, have been used to investigate the distribution, morphology, and potential function of macrophages and the role of inflammation after cochlear insults. Our review of the published evidence shows that macrophage populations across the anatomical regions of the cochlea respond, albeit variably, to tissue injury induced by noise but many of the experimental designs are multifactorial. A challenge, in drawing clear conclusions about the role of macrophages, is the heterogeneity in experimental design between studies, even when considering the consequences of a single type of insult or injury (summarized in Table 2).

3.1 | Variation in noise exposure study design

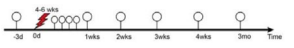
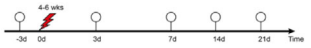
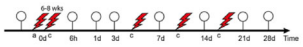
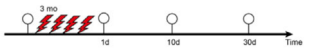
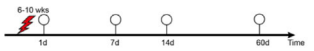
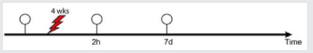
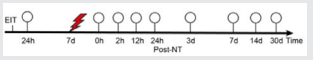
Differences in study design include intensity and duration of noise exposure, age, and strain of mouse, whether the noise exposure caused temporary or reversible threshold shifts and frequency and type of measurements taken. Many of the studies used young mice (4–6 weeks). The immune/macrophage response is likely to be different between older and younger mice since regional, age-dependent differences have been identified in microglial phenotypes between young (4 months) and aged (21 months) C57/BL6 mice (Hart et al., 2012). This may have translational relevance. The level of noise exposure, both intensity and duration, is variable; whereby a significant exposure such as 4 h at 118 dB for four consecutive days, designed to induce long-lasting auditory damage and result in a permanent threshold shift (Fuentes-Santamaria et al., 2017), is likely to elicit a very different inflammatory response compared to lower level noise exposure that only produces temporary threshold shifts (Frye et al., 2018). Although this may be further altered by the age, at which the subject is exposed to the noise or insult (Quraishe et al., 2019). Higher

TABLE 1 The distribution of macrophages across the sub-structures of the cochlea and auditory nerve

Peripheral auditory system								
Anatomical region	**Auditory nerve (AN)**	**Rosenthal's canal (RC)- spiral ganglion neurons (SGNs)**	**Osseous spiral lamina (OSL)**	**Organ of corti (OC)**	**Basilar membrane (BM)**	**Scala tympani (ST)**	**Spiral ligament (SL)**	**Stria vascularis (SV)**
Under homeostatic conditions	Elongated Iba1+ cells with filamentous processes that have terminal podosomes attach to adjacent neurons in the AN[1]***(H)***.	Macrophages with a small cell body with ramified long processes[2]*(R)*. Multiple macrophages amongst the SGNs. Iba1+ cells with pseudopodia and cell projections are observed between the satellite glial cell layer and nerve soma[3]***(H)***. Density of macrophages is positively correlated with the density of spiral ganglion cells[4]***(H)***. Iba1+ cells with a ramified, branching morphology with spider-like extensions[5]*(H)*.	CD163+ and Iba1+ cells are present along OSL[6]***(H)***. Iba1+ and CD68 + present in the OSL[5]***(H)***.	Distinct macrophage populations present during the postnatal developmental period[7]***(M)***. Macrophages are rarely observed under homeostatic conditions[8,9]*(M)*. Human OC contains some active, dendritic, lysosome containing macrophages[1,3,6]***(H)***.	Distinct macrophage populations present during the postnatal developmental period[7]***(M)***. Apical vs basal differences in morphology[10]***(M)***. Located on the perilymphatic side of the BM. Some processes penetrate through BM[4]***(H)***. Located below inner hair cells and at the base of the Hensen cells[6]***(H)***. Few cells present[5]***(H)***.	Macrophages (some PVM/Ms) are observed along the ST wall, usually amoeboid morphology. The cells are CD163+, Iba1+ and CD68 + [6]***(H)***.	Few macrophages observed, often around blood vessels[3]***(H)***. CD163+ and Iba1+ cells are observed amongst various types of fibrocytes[6]***(H)***.	PVM/Ms have branched morphology[11,12]***(M)***. PVM/Ms are tightly associated with endothelial cells[13]***(M)***. PVM/Ms are located near and around blood vessels and intermediate cells[3]***(H)***. Iba1+ and CD68+ cells present in SV[5]***(H)***.

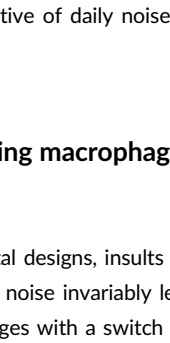
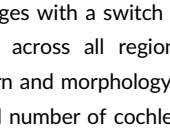
Note: This table summarizes key findings by anatomical location from studies of macrophage distribution and morphology in the cochlea and auditory nerve under homeostatic conditions, that is, in the absence of a deliberate or identified insult. The data are from studies in mouse, rat, and humans. Homeostatic macrophages exhibit a branched, ramified morphology, whereas activated macrophages exhibit an amoeboid morphology, with fewer processes. This morphological characterization of macrophages is indicative, though not definitive, of cellular function. More detailed characterization of the cellular expression patterns and mediator release from the cells is needed to understand macrophage function and heterogeneity. References: 1, (Liu et al., 2019) (H), 2, (Fuentes-Santamaria et al., 2017) (R), 3, (Liu et al., 2018) (H), 4, (Okayasu et al., 2020) (H), 5, (Noonan et al., 2020) (H), 6, (O'Malley et al., 2016) (H), 7, (Dong et al., 2018) (M), 8, (Hirose et al., 2005) (M), 9, (Yang et al., 2015) (M), 10, (Frye et al., 2017) (M), 11, (F. Zhang et al., 2013) (M), 12, (W. Zhang et al., 2012) (M), 13, (Neng et al., 2015) (M). Key: (H) human primary study, (M) mouse primary study, (R) rat primary study.

TABLE 2 Summary of study designs investigating the effect of a single type of insult on macrophages in the auditory system

	Citation	Animal	Strain	Gender	Age	Intensity	Readout/measurement
1	(Hirose et al., 2005)	Mouse	CBA/CaJ	M	8–10 weeks	0, 106, 112, 120 dB	
2	(Sautter et al., 2006)	Mouse	Tg CCL2$^{-/-}$CX3CR1$^{+/GFP}$ & CCR2$^{-/-}$CX3CR1$^{+/GFP}$	M and F	8–10 weeks	0, 106, 112 dB	
3	(Fujioka et al., 2006)	Rat	Sprague Dawley	M	4–6 weeks	124 dB, 4 kHz, 2 h	
4	(B. T. G. Tan et al., 2008)	Mouse	C57BL/6J (M) GFP Tg (F)	M and F	7 months	120 dB, 2 h	
5	(Sato et al., 2008)	Mouse	C57BL/6 & CX3CR1 mutant		10 weeks	112 dB 8–16 kHz, 2 h	
6	(Wakabayashi et al., 2010)	Mouse	C57BL/6J	M	4–6 weeks	124 dB, 4 kHz, 2 h	
7	(F. Zhang et al., 2013)	Mouse	C57BL/6J	M	6–8 weeks	120 dB, 3 h on day 1 and day 2	
8	(Yang et al., 2015)	Mouse	C57BL/6 J, CBA/CaJ & B6.B10ScN-Tlr4lps-del/JthJ	M	4–8 weeks	120 dB, 1–7 kHz, 1 h	
9	(Yang et al., 2015)	Mouse	C57BL/6J	M and F	4–8 weeks	120 dB, 1–7 kHz, 1 h	
10	(Vethanayagam et al., 2016)	Mouse	C57BL/6 J & B6.B10ScN-Tlr4lps-del/JthJ	M and F	4–6 weeks	120 dB, 1–7 kHz, 1 h	
11	(W. J. T. Tan et al., 2016)	Mouse	C57BL/6	M	6–8 weeks	55–65 dB, Acute: 100 dB 24 h Chronic: 90 dB, 2 h per day	
12	(Mizushima et al., 2017)	Mouse	C57BL/6	F	6–10 weeks	121 dB, 4 kHz, 4 h	
13	(Fuentes-Santamaria et al., 2017)	Rat	Wistar	F	3 months	118 dB, 4 h, 4 consecutive days	
14	(Frye et al., 2018)	Mouse	CBA/CaJ	M and F	1–3 months	LLN: 95 dB, 8–16 kHz, 8 h on 16 h off. TN: 120 dB, 1–7 kHz, 1 h	
15	(Kaur et al., 2018)	Mouse	CX3CR1$^{1/1}$ CX3CR1$^{GFP/1}$, CX3CR1$^{GFP/GFP}$	M and F	6–8 weeks	120 dB, 2 h	
16	(Kishimoto et al., 2019)	Mouse	Csf $^{op/op}$ w/ B6C3F1	M and F	4 weeks	120 dB, 8 kHz, 1 h	
17	(Kaur et al., 2019)	Mouse	C57BL/6 (B6) & CX3CR1$^{+/+}$ CX3CR1$^{GFP/+}$ CX3CR1$^{GFP/GFP}$	M and F	6 weeks	90 dB, 8–16 kHz, 2 h	
18	(Mittal et al., 2017)	Guinea pig		M and F		120 dB 6–10 kHz centred at 8 kHz, 2 h	

(Continues)

TABLE 2 (Continued)

	Citation	Animal	Strain	Gender	Age	Intensity	Readout/measurement
19	(Zhang et al., 2020)	Mouse	C57BL/6J & B6.129P2(Cg) Cx3Cr1^{tm1Litt}/J (aka Cx3cr1$^{GFP/GFP}$)	M and F	4–6 weeks	120 dB SPL 1–7 kHz, 1 h (N2- repeated 20 d after first NE)	
20	(Rai et al., 2020)	Mouse	C57BL/6J	M and F	Before 7 weeks	120 dB SPL, 8–16 kHz, 2 h	

Note: The table below summarizes key methodological variables in published studies of noise damage to the auditory system in which the effect on macrophage populations was assessed. Variables are: age, strain, and gender of animal and also the intensity and duration of noise exposure. The timeline indicates the age at which the animals were exposed to noise and the number of measurements taken across the timeline of the experiment.

intensity exposures may provide insight into the damage caused by repeat exposure to a significant event, for example, a blast, whereas the lower level noise is more representative of daily noise exposure associated with certain industries.

3.2 | Pathophysiology involving macrophages following insults to the cochlea

Despite the heterogeneity of experimental designs, insults or trauma to the cochlea such as exposure to loud noise invariably lead to the activation of resident cochlear macrophages with a switch to a more pro-inflammatory, activated, phenotype across all regions of the cochlea. This change in expression pattern and morphology is associated with changes in the distribution and number of cochlear macrophages, as summarized in Table 3. The resulting inflammatory response is associated with damage and degeneration within cochlear structures including sensory cells (Bohne & Harding, 2000), spiral ganglion neurons (Wang et al., 2002) and cochlear atrophy. Perivascular macrophage-like melanocytes (PVM/Ms) in the stria become activated and change their morphology and distribution, resulting in smaller PVM/Ms with shorter processes, which are physically detached from capillary walls (Neng et al., 2015; Noble et al., 2019; Zhang et al., 2013). PVM/Ms are essential for adequate hearing function but persistent activation of PVM/Ms in response to an inflammatory event causes strial dysfunction. Breakdown, or even a partial/transient loss of integrity of the blood-labyrinth barrier, including the intrastrial blood-fluid barrier, enables leakage of molecules and cells from the circulation and contributes to altered or lost hearing.

Exposure to noise results in activation and a greater number of macrophages in the spiral ligament (Fuentes-Santamaria et al., 2017), with significant monocyte infiltration (Hirose et al., 2005; Sautter et al., 2006; Tornabene et al., 2006). The macrophages amongst the spiral ganglion neurons become activated; increasing in number with an amoeboid appearance, indicating a switch to a pro-inflammatory, activated, phenotype (Fuentes-Santamaria et al., 2017; Kaur et al., 2018). Similarly, macrophages in the central portion of the auditory nerve increase in number and appear activated (Fuentes-Santamaria et al., 2017). They also display a shift to more variable morphologies with increased age (Noble et al., 2019). Acute noise exposure results in the infiltration of monocytes to the basilar membrane (Yang et al., 2015) and the activation of macrophage populations in regions adjacent to sensory cells (Frye et al., 2018). Whereas aging results in the activation of tissue-resident macrophages with little monocyte infiltration (Frye et al., 2017).

Damaged spiral ganglion neurons (SGNs) and surrounding resident cells produce pro-inflammatory cytokines that activate and recruit macrophages (Bas et al., 2015; Fuentes-Santamaria et al., 2017). There is evidence that these macrophage responses have a neuroprotective role mediated through fractalkine signaling. Selective hair cell ablation, aminoglycoside ototoxicity, and noise trauma result in fractalkine-dependent recruitment of macrophages to the sensory epithelium and spiral ganglion of the cochlea that is

TABLE 3 Changes in macrophage distribution and morphology in the cochlea and auditory pathway following different cochlear insults including noise exposure, aging, and cochlear implantation

Anatomical region	Auditory nerve (AN)	Rosenthal's canal (RC)- spiral ganglion neurons (SGNs)	Osseous spiral lamina (OSL)	Organ of corti (OC)	Basilar membrane (BM)	Scala tympani (ST)	Spiral ligament (SL)	Stria vascularis (SV)
After noise exposure (NE)	Increase in Iba1 staining. Cells resemble activated microglia[2](R).	Activated with amoeboid morphology[2](R). Increase in the number of macrophages[14](M).	Increase in the number of macrophages[15](M).	†	Site of migrating macrophages[8](M). Acute NE - infiltration of monocytes[9](M). Low level NE- macrophage activation to amoeboid morphology. Increased number in regions adjacent to sensory cells[16](M). Increase in the number of macrophages, change from monocyte-like to amoeboid morphology after 20d. Increased number of activated macrophages after second NE[15](M).	Increase in the number of macrophages on the luminal surface[15](M).	Increase in the number of macrophages and the number of activated macrophages[2](R). Significant monocyte infiltration[8,17,18](M).	PVM/Ms change morphology. Physical detachment of PVM/Ms from capillary walls[11](M).
With aging	Number of macrophages increases with age, variable morphologies. Increase in activated macrophages [19](H).	Worm-like cytoarchitecture located near neuronal soma. Increased interactions between macrophages and glial cell-associated myelinated axonal projections of type I SGN cell bodies[19](H).	Bipolar architecture and flat encroaching filopodia-like structures suggesting interactions with peripheral neural projections[19](H).	†	Resident tissue macrophages involved in the pathology rather than the infiltrated macrophages. Number of apical macrophages increases, number of basal macrophages decreases[10,20](M).	†	Increase in the number of macrophages in the middle and basal turns. Increase in activated macrophages with amoeboid morphology[19](H).	PVM/Ms are smaller with shorter processes[13](M). PVM/Ms are highly ramified in aged human temporal bones[19](H).
After cochlear implantation	Increase in the infiltration of macrophages following implantation [21](M).	Monocyte invasion increases rapidly after implantation[21](M). Density of macrophages is greater in the basal RC. Transitional, ramified morphology with long processes. Increased number of macrophages 'wrapping' spiral ganglion cells[4](H).	Higher density of macrophages. More amoeboid morphology[4](H).	High IL-1β levels on day 14 which then remained stable until day 30 - pro-inflammatory environment[21](M). No difference in the distribution of macrophages in the OC after 12 to 210 months after implantation[4](H).	Few macrophages observed beneath the OC with processes extending into the OC[4,5](H).	Iba1+ macrophages within areas of fibrosis in the scala tympani[5](H).	No difference observed in the number of macrophages [4](H).	PVM/Ms observed near blood vessels. Increased infiltration of monocytes over time[21](M). No difference in the number of macrophages [4](H).

Note: The table summarizes the changes in distribution, morphology and abundance of macrophages following cochlear insults such as noise exposure, aging and cochlear implantation. Inflammation following cochlear insults is often associated with an increase in number and activation status of cochlear macrophages. These changes reflect the role of these cells in the tissue injury and repair response. There is evidence from other pathologies that dysregulated macrophage responses are associated with change or loss of function. Key: † No reporting on this to be found, (H) human primary study, (M) mouse primary study, (R) rat primary study. References: 2, (Fuentes-Santamaria et al., 2017) (R), 14, (Kaur et al., 2018) (M), 15, (C. Zhang et al., 2020) (M), 8, (Hirose et al., 2005) (M), 9, (Yang et al., 2015) (M), 16, (Frye et al., 2018) (M), 17, (Sautter et al., 2006) (M), 18, (Tornabene et al., 2006) (M), 11, (F. Zhang et al., 2013) (M), 19, (Noble et al., 2019) (H), 10, (Frye et al., 2017) (M), 20, (Frye et al., 2018) (M), 13, (Neng et al., 2015) (M), 21, (Bas et al., 2015) (M), 4, (Okayasu et al., 2020) (H), 5, (Noonan et al., 2020) (H).

associated with survival of the spiral ganglion neurons (Kaur, Zamani, et al., 2015b; Kaur et al., 2018, 2019).

An understanding of the precise molecular mediators that influence the macrophage phenotype after a cochlear insult is lacking. Collating information from multiple (animal and human) studies, as displayed in Table 3, to begin to build a consensus understanding of macrophage morphology, distribution, and function across different regions of the cochlea after cochlear insults will enable better understanding of how these cells respond to cochlear insults and potentially contribute to an individual's hearing loss trajectory. Further investigation into other immune insults that could occur throughout a lifespan, such as peripheral and systemic infection and inflammation, and which may influence the function and behavior of cochlear macrophages is necessary.

4 | IMMUNE EVENTS FOLLOWING AN ACUTE INSULT TO THE COCHLEA

Acute insults to the cochlea include noise exposure (Frye et al., 2018; Hirose et al., 2005; Sato et al., 2010), ototoxicity (Kaur et al., 2018; Sato et al., 2010), selective hair cell ablation (Kaur, Zamani, et al., 2015b) and cochlear implant surgery (Bas et al., 2015; Seyyedi & Nadol, 2014). These lead to the rapid onset of sensory cell injury and tissue pathogenesis. These signals to resident cochlear macrophages, switching them to an activated, pro-inflammatory state. Pro-inflammatory macrophages release cytokines such as TNF-α (Keithley et al., 2008) and chemokines including CCL2 (Bas et al., 2015; Sautter et al., 2006; Zhang et al., 2020) which attract and recruit inflammatory, circulating monocytes into the cochlea (Frye et al., 2017; Hirose et al., 2005; Okayasu et al., 2020; Tan et al., 2008; Vethanayagam et al., 2016; Yang et al., 2015). These infiltrated monocytes differentiate into macrophages, in the cochlea, and carry out characteristic immunological functions including phagocytosis (Kaur, Hirose, et al., 2015a), inflammatory mediator production, and antigen presentation (Yang et al., 2015).

4.1 | Time course of macrophage activity

The time course of monocyte infiltration following acute injury is variable, depending on the type, and duration of injury. For example, infiltration beginning between 12 and 24 h, has been described after noise exposure, with a peak at 3–7 days and then a gradual decrease as the inflammation appears to resolve (Hirose et al., 2005; Tornabene et al., 2006; Wakabayashi et al., 2010). By contrast, a continuous presence of monocyte-like macrophages ($CD45^{high}$, $F4/80^{low}$, $Iba1^{low}$) in the cochlea at 20 days after noise exposure has been observed (Zhang et al., 2020). Some of these macrophages have been identified as adopting an anti-inflammatory profile ($Ly6C^{low}$), consistent with a healthy tissue reparative response, where an acute wave of inflammatory activity is closely followed by resolving, anti-inflammatory signaling. Following cochlear implantation, monocyte infiltration to the wound-site increases rapidly at 1 day post-implantation with peak numbers detected at 7 days post-implantation (Bas et al., 2015). The precise role and turnover rate of the newly infiltrated macrophages is not well understood, though there is evidence that some of these cells persist (Okano et al., 2008; Sato et al., 2008). Although cochlear implantation is a single event, the signaling and tissue response may persist to a variable degree. Surgical insertion of an electrode array inside the cochlea causes physical trauma. The inflammatory response to this is likely as has been described above and should resolve as part of a normal wound healing response. However, in some individuals there is a chronic inflammatory response, which promotes the excessive deposition of fibrotic tissue around the array, which develops long after the surgical intervention, leading in some cases to reduced performance with the device (Bas et al., 2015; Hough et al., 2021; Nadol et al., 2008, 2014).

5 | IMMUNE EVENTS ASSOCIATED WITH A CHRONIC INSULT TO THE COCHLEA

Chronic damage/deterioration of the auditory system, such as that due to aging, chronic noise exposure, or after cochlear implantation is thought to trigger a different immune response to an acute event. The effects of cumulative low-grade cochlear insults, such as can occur with aging, have been investigated in a small number of studies (Frye et al., 2017; Noble et al., 2019; Su et al., 2020; Zhang et al., 2017). Cumulative, progressive, sensory cell degeneration and death leads to the activation of resident macrophages, with little infiltration of circulating monocytes (Frye et al., 2017), which parallels the innate immune response seen in chronic compared to acute CNS disease. Activation of resident macrophages is associated with a morphological change to macrophages with a larger cell body and amoeboid shape. The activated, resident macrophages adopt either a pro- or anti-inflammatory profile. Pro-inflammatory macrophages produce and release pro-inflammatory mediators including IL-1β, TNF-α, IL-6, which signal to surrounding cells resulting in further inflammation and cellular damage/apoptosis (Fujioka et al., 2006; Tan et al., 2016; Wang et al., 2019a). Anti-inflammatory macrophages produce anti-inflammatory, tissue reparative, mediators such as IL-10 and arginase (Kalinec et al., 2017).

5.1 | Inflammaging and progressive hearing loss

Chronic inflammation or 'inflammaging' describes low-grade inflammation that can occur in aging tissues and that worsens with age (Franceschi et al., 2006, 2017; Gruver et al., 2007; Watson et al., 2017). It is associated with age-related changes in immune function, characterized by a decreased ability to control the production of pro-inflammatory proteins, which results in an increased inflammatory state in the majority of tissues. Data from cross-sectional aging cohort studies showed that higher levels of circulating markers of inflammation are associated with a more marked age-related hearing loss

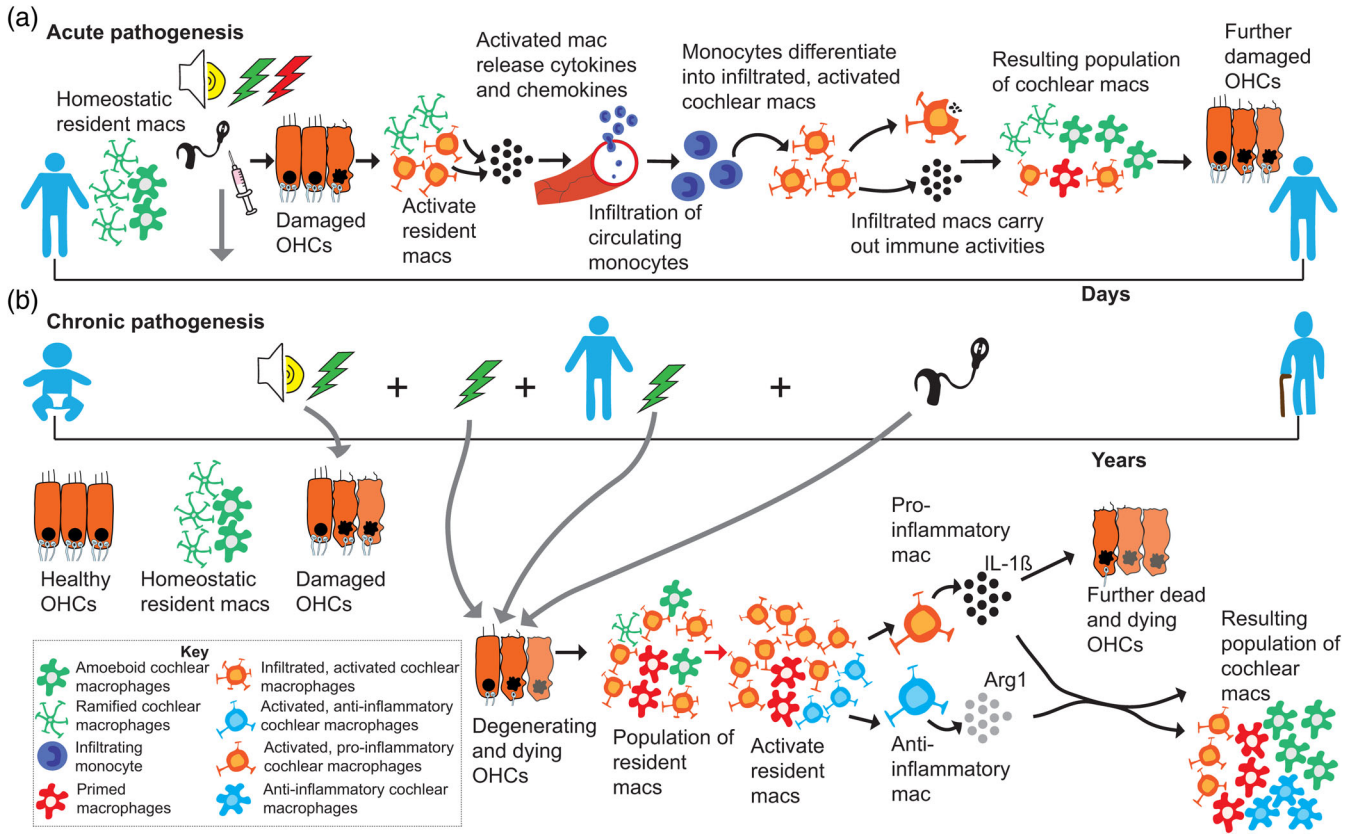


FIGURE 4 A role for macrophage activation in response to acute and chronic insults in the cochlea. A summary of the sequence of immune events that occur in the cochlea following (a) an acute insult and (b) how this may be altered in the context of chronic insults such as repeated noise exposure or age-associated changes, if activated cochlear macrophages become primed. (a) Multiple studies have shown that an acute insult with sensory cell and tissue injury signals to resident cochlear macrophages (and cochlear fibrocytes) resulting in their activation. Activated, pro-inflammatory cochlear macrophages and fibrocytes release cytokines and chemokines which recruit circulating bone-marrow derived monocytes. On entry to the cochlea, the monocytes differentiate into activated macrophages. Further tissue damage may occur due to local release of mediators from these macrophages resulting in cellular dysfunction and hearing loss. Typically, a wave of inflammation is followed by induction of an anti-inflammatory response that results in resolution of the inflammatory response and tissue-remodeling. However, some macrophages may adopt an altered or primed phenotype in response to the initial activating insult. (b) Chronic, cumulative, progressive insults such as repeated noise exposure and/or age cause gradual sensory cell denervation and degeneration. This signals to activate resident cochlear macrophages to express a pro-inflammatory profile and secrete pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α. This activation may result in a switch to a primed state, such that the macrophages develop a lower threshold (hyper-responsiveness) to subsequent insults, whereby they mount a more florid inflammatory response, with greater numbers of cells with greater associated tissue damage. Tissue damage in the auditory system is likely to manifest as a progressive loss of hearing function. The precise aspect of the function that is impaired reflects the site within the system that has been injured. References: (a) (Bas et al., 2015; Fuentes-Santamaria et al., 2017; Hirose et al., 2005; Kaur, Zamani, et al., 2015b; Okano et al., 2008; Shi, 2010; Yang et al., 2015) (b) (Frye et al., 2017, 2018; Noble et al., 2019; Zhang et al., 2020)

(ARHL) (Bainbridge et al., 2010; Nash et al., 2014; Simpson et al., 2013; Verschuur et al., 2014; Verschuur et al., 2012). Similarly, data from a longitudinal aging study showed that elevated white blood cell count was positively correlated with degree of hearing loss in older adults (Lassale et al., 2020). A small longitudinal population study from our own group (unpublished 57 adults aged 65–75 at time of recruitment) identified an association between increased activation of macrophages, as detected by the excretion of a metabolite (neopterin) and the likelihood of more marked low frequency hearing loss. The greater the number of times across a 12-month period neopterin was elevated above the basal rate for adults, the greater was the likelihood of more marked low frequency hearing loss 3–4 years later. Much like other organs, the immune system within the auditory system is exposed to multiple insults, such as the effects of co-morbidities across the lifespan. The combination of type, intensity, and temporal sequence of insults, or immunobiography, is unique for each individual (Franceschi et al., 2017; Haley et al., 2018; Netea et al., 2020).

5.2 | Cumulative effect of cochlear insults

The cumulative effect of cochlear insults is not well understood. Investigation of the interactions between noise-induced hearing loss (NIHL) and age-related hearing loss (ARHL) found that early life noise exposure made the cochlea more vulnerable to changes commonly

associated with aging (Kujawa & Liberman, 2006). A widespread loss of spiral ganglion cells (not associated with hair cell death) was seen in older mice that had been noise-exposed that was not evident in unexposed age-matched controls (Kujawa & Liberman, 2006). Evidence from a repeated noise model highlighted the effect of prior macrophage activation on the cochlear response to subsequent acoustic trauma (Zhang et al., 2020). In mice exposed to 120 dB SPL noise for 1 h then re-exposed 20 days later, a greater increase in macrophage populations in the osseous spiral lamina, luminal surface of the scala tympani, and underneath the basilar membrane was measured compared to cell numbers in those regions following only the first noise exposure (Zhang et al., 2020). Additionally, after the second noise exposure (or insult) there was a significant increase in the number of macrophages with an amoeboid morphology; suggestive of a transition to a more pro-inflammatory phenotype following an initial exposure. This is the first published study to closely examine the macrophage distribution, morphology, and phenotype following repeated insults alongside providing evidence for an exaggerated inflammatory response following a prior immune activation in the cochlea (Zhang et al., 2020). The mechanistic links between noise induced hearing loss (NIHL) and age-related hearing loss (ARHL) (Eckert et al., 2021; Mills et al., 1997) are not yet understood but macrophages and their ability to retain immunological memory are a putative link. This is supported by the evidence from the studies that identify the distribution and morphology of macrophages (summarized in Tables 1 and 3) and the immune response to acute and chronic tissue changes seen in NIHL, ARHL, and following cochlear implantation (Figure 4).

5.3 | Priming of tissue-resident macrophages

Microglia, once activated, can persist in a hyper-responsive, pro-inflammatory state making them more susceptible to a secondary inflammatory stimulus, which can trigger a subsequent exaggerated inflammatory response (Perry & Holmes, 2014). The nomenclature of the different activation states of microglia and macrophages is the subject of ongoing discussion in the literature (Haley et al., 2018; Neher & Cunningham, 2019; Perry & Teeling, 2013). The term "primed" is applied to microglia with an immunophenotype associated with memory of previous insults. There is overlap between *priming* and the term *trained*, which is used to describe this state in macrophages in other organ systems. Primed microglia typically show greater expression of inflammatory markers coupled with a lower threshold to switch to a pro-inflammatory state (Lull & Block, 2010). Once triggered, they exhibit an exaggerated inflammatory (with associated local tissue damage) response to immune activation (Lull & Block, 2010; Norden et al., 2015). Primed microglia have been described in models of aging, neurodegenerative disease, and traumatic brain injury (Cunningham et al., 2005; Kokiko-Cochran & Godbout, 2018; Lopez-Rodriguez et al., 2021; Neher & Cunningham, 2019). However, priming of microglia/macrophages in the auditory system has not been described in the literature, despite the similarities in macrophage responses described within the auditory system.

Similarities can be drawn between how macrophages/the immune system respond to insults across different organ systems. Traumatic brain injury (TBI) is defined as the functional disruption of the brain from an impact (blunt) or penetrating injury (Centers for Disease Control and Prevention, 2013). Typical pathological features of TBI include BBB disruption, neuronal and axonal injury, oedema, focal contusion and a widespread inflammatory response including microglial and astrocyte activation, infiltration of peripheral cells and increased production and release of inflammatory molecules (Puntambekar et al., 2018). Many of these pathological features are similar to that following an insult, such as exposure to loud noise, to the cochlea or elsewhere in the auditory pathway.

Figure 4 integrates the findings from key studies and the sequence of immune events that take place in the cochlea during acute and chronic pathogenesis. The presence of primed macrophages has been added based on the hypothesis proposed through this review. The timeline emphasizes the differences in time course between acute (across days) and chronic (across years/lifetime) pathogenesis. This allows for current and future studies to be put into context of where an experimental design sits in relation to the relative contribution of acute and chronic insults, the interaction between both, and the consequences of these insults on immune cell population and tissue damage.

5.4 | Proposing priming of cochlear macrophages

The timeline depicted in Figure 4b demonstrates that a single, acute insult (such as noise exposure) causes damage to OHCs. However, this is happening alongside the accumulation of multiple insults over time, leading to the development of chronic pathology, which involves the slow, progressive degeneration of the sensory cells and the activation of the long-lived resident cochlear macrophages. Based on the understanding of glial biology from other systems, we propose that some of the macrophages involved in this response are likely to have already been exposed to an immunological insult, or been trained, and have therefore adopted a primed, or more responsive state. Upon activation through further cochlear insults or through chronic inflammation associated with aging and age-associated morbidities (which are also risk factors for hearing loss), these macrophages may exhibit an exaggerated inflammatory response and exacerbate cochlear damage and manifest as progressive hearing loss (as depicted through the schematic in Figure 5).

6 | DO MACROPHAGES INFLUENCE THE TRAJECTORY OF HEARING LOSS?

Macrophage phenotype including morphology, expression of markers and the signals they produce and release, the way in which macrophages behave and how they respond to inflammatory insults, play a significant role in both normal and pathological auditory function across a lifetime. Insults, or injury to the cochlea, such as noise

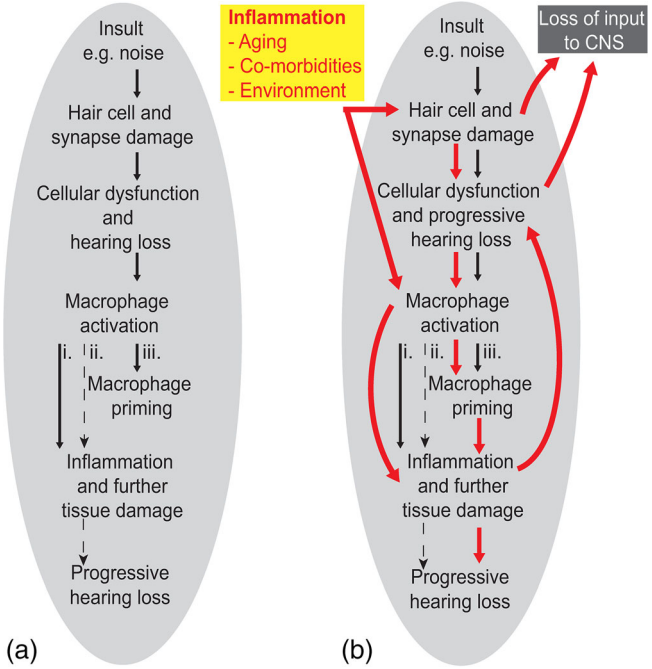


FIGURE 5 Schematic of the hypothesis proposing the role for cochlear macrophages in contributing to the pathology of hearing loss. (a) An insult to the auditory system such as noise overexposure, causes hair cell and synapse damage in the cochlea which results in cellular dysfunction and hearing loss. Damaged sensory cells release mediators which recruit and activate resident cochlear macrophages (i). Activated macrophages will adopt a pro-inflammatory or anti-inflammatory phenotype. Pro-inflammatory macrophages produce and release pro-inflammatory cytokines and chemokines, resulting in inflammation and cellular damage or apoptosis. (ii). Some macrophages may already be in a pro-inflammatory state from a previous insult (primed), therefore upon activation display an exaggerated inflammatory response resulting in further inflammation and damage to tissues in the local area, which will contribute to progressive hearing loss. (iii). Some macrophages may switch to a primed phenotype as a result of this insult. (b) Additional inflammation due to aging, co-morbidities or environmental factors results in further hair cell and synapse damage (and subsequent progressive hearing loss) and further macrophage activation. (iii). Macrophages in an active, pro-inflammatory state as a result of the previous noise exposure may be primed and therefore display an exaggerated inflammatory response to the additional inflammation, causing further tissue damage and progressive hearing loss. Sensory cell and tissue damage and the resulting loss of function of essential cells in the cochlea, causes loss of input to the central auditory pathways. Decreased activation of the ascending and descending central auditory pathway leads to dysfunctional auditory processing

exposure, the chronic deterioration of the auditory system that occurs with aging (Jafari et al., 2019) and conditions causing systemic inflammation are associated with a greater degree of hearing loss and the evidence suggests that the accumulation of insults such as early noise exposure plus overlaid on the effects of aging (Kujawa & Liberman, 2006) or repeated noise exposure (Zhang et al., 2020) may exacerbate this hearing loss. Despite this, the mechanisms responsible for the pathology causing hearing loss are not well understood although inflammation and cochlear macrophages are involved in its pathogenesis.

6.1 | Evidence of priming/innate immune memory in models of disease/aging

Based on the understanding from the fundamental studies discussed in this review and our wider understanding of how macrophages respond to inflammatory insults in chronic conditions such as traumatic brain injury (TBI), Alzheimer's disease (AD), and age-associated changes, we hypothesize that innate immune memory and the priming of cochlear macrophages and microglia in the auditory pathway influences the trajectory of hearing loss.

Priming was first observed in peritoneal macrophages exposed to lipopolysaccharide (LPS). Isolated macrophages produced increased IL-1 and inducible nitric oxide synthase (iNOS) (relative to non-primed macrophages) if the cells were pre-exposed to or 'primed by' interferon gamma (Pace et al., 1983). A study by Cunningham et al demonstrated this priming effect in microglia, where an enhanced immune response in the ME7 prion mouse model of chronic neurodegeneration was measured after administration of LPS. Either direct challenge of the CNS with LPS, or a systemic challenge with LPS, resulted in increased microglial activation and greater inflammatory response in the prion mice compared to wild-type animals (Cunningham et al., 2005). Similar observations have been described in models of aging (Godbout et al., 2005), Wallerian (axonal) degeneration (Palin et al., 2008) and Alzheimer's disease (Holtman et al., 2015; Lopez-Rodriguez et al., 2021).

The proposed interaction between chronic immune state and new acute insults to the auditory system is supported by evidence that the inflammatory response to TBI is influenced by previous and subsequent inflammatory challenges (Kokiko-Cochran & Godbout, 2018), potentially through the mechanism of microglial priming. To date, there are no effective interventions to reliably improve outcome after TBI. A variety of TBI models are being used to investigate the mechanisms and pathology, however, due to the different types and the complexity of TBI, and the array of primary and secondary damage which occurs in human TBI, no single model fully recapitulates all aspects of human TBI. Despite the complexity of understanding these overlapping but distinct mechanisms, all TBI models show the consistent involvement of a microglia/macrophage inflammatory response highlighting these cells as critical mediators in the outcome.

6.2 | Utilizing the auditory system to investigate macrophage biology

Noise injury at a specific volume and frequency can be a traumatic injury to the cochlea and auditory system resulting in a measurable loss of function, which is experimentally reproducible (Fuentes-Santamaria et al., 2017; Kurabi et al., 2017; Zhang et al., 2020). Using a frequency-specific noise to create an injury in the cochlea, it is

possible to reliably map the injury site, due to tonotopy of the cochlea. While with noise injury there is a small frequency shift, this shift is reproducible (Quraishe et al., 2019). The resulting tissue damage and the local cellular response from the insult, in and around the area of damage, can be investigated. Functional readouts following the injury can be obtained to determine the severity of damage to the auditory system and any subsequent additional (or progressive) hearing loss. Significantly, there is also the option to study the effect of a loss and re-establishment of function in the auditory system through the use of hearing aids or cochlear implants. Therefore, utilizing the auditory system to investigate macrophage/microglial priming may be beneficial to inform how these cells behave in other pathologies/diseases.

6.3 | Can macrophages contribute to the individual trajectory of hearing loss through priming?

The evidence is that inflammatory insults induce innate immune memory (trained immunity) and that exposure to insults over time establishes an individual's immunobiography. This influences how they respond to further immune insults across their lifespan. In terms of the auditory system, the integration of immunobiography, trained immunity, and inflammaging may explain why individuals exhibit heterogeneity in their hearing-loss trajectory as they go through life, including both their natural hearing alone or with the addition of a hearing aid or cochlear implant. Macrophage priming/training may occur if the cell has been previously activated and returned to a quiescent state but retained a 'memory' of the event so that it produces exaggerated acute responses to subsequent challenges. Persistent cochlear inflammation may result in a chronic state of activation of macrophages, resulting in a persisting phenotype that is primed. A better understanding of the mechanisms by which trained immunity may contribute to chronic inflammaging with aging, and what factors may exacerbate cochlear inflammation, may enable lifestyle or pharmaceutical interventions to slow down the decline in hearing function with age.

7 | CONCLUSION

This review synthesizes the current knowledge of the distribution, phenotype, and function of macrophages under both homeostatic and pathological conditions in the cochlea. Following an acute insult, which can lead to sensory cell and synaptic damage, cochlear macrophages become activated and initiate a typical immune response involving both pro-inflammatory and anti-inflammatory signaling. The macrophage response and molecular mediators involved in their function following progressive, cumulative insults over time needs further investigation. We propose, based on the evidence from other organs in the body, that macrophages contribute to the progressive loss of hearing experienced by many people worldwide and to the variability in hearing function that exists between individuals. Out of necessity, and reflecting the field, much of the evidence is from studies in mice though we have included human data where available. Greater focus on fundamental macrophage biology in the human auditory system will enable advances in understanding of the mechanisms involved in hearing and therefore how to target these mechanisms to improve hearing outcomes for people with hearing loss.

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DATA AVAILABILITY STATEMENT

The majority of the data referred to in the manuscript is already in the public domain, tables that have been assembled during the process of the detailed review of the literature have been included. The data referred to in the unpublished study from our group describing evidence of macrophage activation being associated with hearing loss is being prepared for publication.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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