Immune Mechanisms of the Inner Ear

Elizabeth M. Keithley, Ph.D.

UCSD Dept of Otolaryngology, Head & Neck Surgery, La Jolla, CA
Why study Cochlear Immune Responses?

To understand hearing in all its complexity

Defining cochlear immune responses and how they are regulated will lead to better therapies for hearing loss

Anti-inflammatory drugs can restore hearing in some patients with suspected immune-mediated hearing loss
Immune function involves interaction among:

- Inner Ear
- Immune cells
- Systemic circulation – vasculature and lymphatics
- Lymph nodes & diffuse lymphoid tissues
- Bone marrow
- Thymus
- Spleen
Classic Immune Categories

Abbas et al., 2007
Tissue Resident Macrophages

Multifunctional supervisory cells involved in homeostasis and tissue repair

Monitor tissue environments to initiate and control the appropriate cell signaling cascades

Pleomorphic and capable of changing shape and migrating in response to environmental signals

Diverse gene expression patterns across and within organs
Inner Ear Resident Macrophages

Cochlea: Stria Vascularis
  Spiral ligament
  Scala tympani – periosteum & basilar membrane
  Modiolus – limbus, osseus spiral lamina
  Round window membrane

Vestibular macula, saccule, utricle

Endolymphatic duct and sac
Ionized calcium-binding adaptor molecule 1 (Iba1)-positive macrophages

Stria Vascularis and Otic Capsule
Stria Vascularis

CD31/IBA1

IBA1⁺ macrophages
P14 mouse
whole-mount prep

Dr. Hainan Lang, MUSC
(unpublished)
Macrophages in Stria Vascularis

Xiaorui Shi, 2016. Oregon Health and Science University
Spiral Ligament

mouse
Anti-F480

Masumichi Miyao

Human
Spiral Ligament
Anti-Iba1

Jennifer O’Malley and Mike McKenna
Basilar Membrane

mouse

Anti-F480

Antic-CD45

apical

middle

basal

Hu, Zhang, and Frye, 2018
Sensory Epithelium

human

SM

ST

Jennifer O’Malley and Mike McKenna
Sensory Epithelium

human

ST

Jennifer O’Malley and Mike McKenna
Macrophages and Hair Cell Degeneration

Dying HC are phagocytosed by Deiter’s cells.
Reticular membrane sealed as HC is eaten. (Anttonen et al., 2014; Lee et al., 2021)

HC and supporting cells express cytokines, chemokines, and other immune mediators
Modiolus ganglion, nerve, modiolar artery & vein, osseus spiral lamina & limbus

Anti-Iba1-Human cochlea

Jennifer O’Malley and Mike McKenna
Modiolus – Spiral Ganglion

Anti-Iba1-Human spiral ganglion

Jennifer O’Malley and Mike McKenna
Vestibular End-organs: utricle, saccule & semicircular canal ampullae

Macrophages are present within the neurosensory epithelia and supporting, stromal layer

Experimental damage to sensory cells increases number of macrophages (Kaur et al., 2015)

Macrophages phagocytose dying sensory cells (Kaur et al., 2015)
Endolymphatic Duct and Sac

Anti-Iba-1 stained human, celloidin-embedded section

Jennifer O’Malley and Mike McKenna
Inner Ear – perilymph and endolymph

Endolymphatic Duct and Sac

Lining cells are continuous with those lining the e. space.
Subepithelial connective tissue is continuous with perilymphatic space.
Bone marrow is adjacent to e. sac.

Lumen contains normal, high Na+ fluid, macrophages, lymphocytes, plasma cells and cellular debris.
Connective tissue contains monocytes, macrophages, T and B lymphocytes, plasma cells, PMNs, blood vessels and fenestrated vessels likely lymphatic vessels.
Immune Relationship between Endolymphatic Sac and Cochlea

Ag injected into the scala tympani reaches the e. sac and is phagocytosed.

Bacteria injected Intrathecally reach the e. sac through the cochlear aqueduct and are phagocytosed.
Relationship between Endolymphatic Sac and Cochlea

Experimentally induced inflammation in the endolymphatic sac causes:

- ICAM-1 expression in spiral ligament fibrocytes and vascular endothelial cells,
- Extravasation of leukocytes into the cochlea
- Endolymphatic hydrops in the cochlea.

Surgical destruction of the e. sac reduces the magnitude of experimental cochlear inflammation.
Experimentally induced inflammation in the cochlea causes:

- ICAM-1 expression in e. sac venules

- Expression of the pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α in some e. sac inflammatory cells
Relationship between Endolymphatic Sac and Cochlea

IL-1β expression induced by cochlear immune response

Satoh et al., 2003
Relationship between E. Sac and Cochlea

The endolymphatic sac tissue with its vascular, lymphatic and immune cells operates in a coordinated manner with the inner ear immune responses.

Tomiyama and Harris, 1986, 1987, 1989
Satoh et al., 2003
Communication Between the Inner Ear and Systemic Immune System

Vasculature / Circulation
Lymphatics
Inflammatory cells enter ST from systemic circulation
Inflammatory cells enter ST from systemic circulation

Immune / inflammatory cells interact with extracellular matrix molecules (proteins and proteoglycans).

Fluid-filled perilymph is a unique environment. As immune cells enter the scalae, matrix is generated. By what cells?

The matrix can become ossified. Always? How regulated?
Lymphatic Drainage of Inner Ear

Fate of Ag injected into the Cochlea

Yimtae et al., 2001
Ag – labeled deep cervical lymph node – 15 minutes after cochlear injection

Yimtae et al., 2001
Ag – labeled spleen cells 15 minutes after cochlear injection

Yimtae et al., 2001
Communication Between the Inner Ear and Systemic Immune System

Antigens and pathogens that enter the inner ear from the CNS, via the cochlear aqueduct, middle ear, or the round window membrane, are quickly processed by the systemic immune system.
Activation of Innate Immunity by LPS

Lipopolysaccharide = endotoxin = LPS

Cell wall component of gram-negative bacteria, released from dying bacteria

Used experimentally to simulate infection

LPS stimulates many innate immune responses including,

1. cytokine secretion
2. adhesion molecule expression on endothelial cells
How does the Cochlea respond to Systemic LPS?

Vascular permeability is increased – Hirose et al., 2014

Type II spiral ligament fibrocytes express NFκB, an innate immune activator - Adams et al., 2009

Type I spiral ligament fibrocytes express IL-1β – Hashimoto et al., 2005

CD45+ leukocytes enter scala tympani – Hashimoto et al., 2005

Noise damage is increased – Herranen et al., 2018
IL-1β Expression in Spiral Ligament Fibrocytes

72 hours after i.p. Injection of LPS

Hashimoto et al., 2005
Cochlear response to Systemic LPS

72 hours after i.p. Injection of LPS

CD-45 immunolabelled – leukocytes in the spiral ligament and scala tympani

Hashimoto et al., 2005
Effect of LPS on Cochlear Adaptive Immune Response

Hashimoto et al., 2005
Conclusion

Activity in the cochlea is constantly monitored by systemic immunity

Conversely, systemic immune activity induced by infection affects the cochlea
Macrophages assist in repair of damaged HC-SGC synapses (Manickam et al., 2023).

Upregulation of the pro-inflammatory mediator, NFκB (Adams et al., 2009).

Secretion of pro-inflammatory cytokines such as: TNF-α, IL-1β and IL-6 and chemokines such as: MCP-1, MCP-5, CCL2

Recruitment of circulating inflammatory cells
Activation of Innate Immunity by Acoustic Trauma

Acoustic Trauma - 8-16 kHz noise, 100 dB for 2 hours. Survival time – 24 hours

Activation of NF-κB, a transcription factor for cytokines, in type I spiral ligament fibrocytes.

Adams et al., 2009
Macrophages in Spiral Ligament - acoustic trauma

F4/80 – activated macrophages

CD-45 pan-leukocyte
Basilar Membrane

Young mice exposed to noise: 8-16 kHz 95 dB SPL

Frye, Zhang & Hu, 2018
Infiltration of Leukocytes after Acoustic Trauma

Cat cochlea, 48 hrs after noise exposure. celloidin embedded. H&E stained

Section courtesy of M. Charles Liberman, MEEI
Effect of Noise on cochlear adaptive immune response

8 days post intrathecal Ag challenge

7 days post noise exposure

Miyao et al., 2008
Conclusions

Inner ear immune activity is an exciting topic of investigation with many unknowns. The inner ear is not isolated from systemic immune activity, but affects it and is affected by it – immune surveillance is a constant occurrence.
Acknowledgments

Jeffrey P Harris
Gary Firestein

Allen F Ryan

Hainan Lang
Jennifer O’Malley

Hitoshi Satoh
Masumichi Miyao
Shigehisa Hashimoto
Peter Billings
Xiaobo Wang
Tim Truong
Kunihiro Sato
Steve Tornabene

NIH NIDCD
Dept of Veterans Affairs