Immune Mechanisms of the Inner Ear

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Why study Cochlear Immune Responses?

To understand hearing in all its complexity

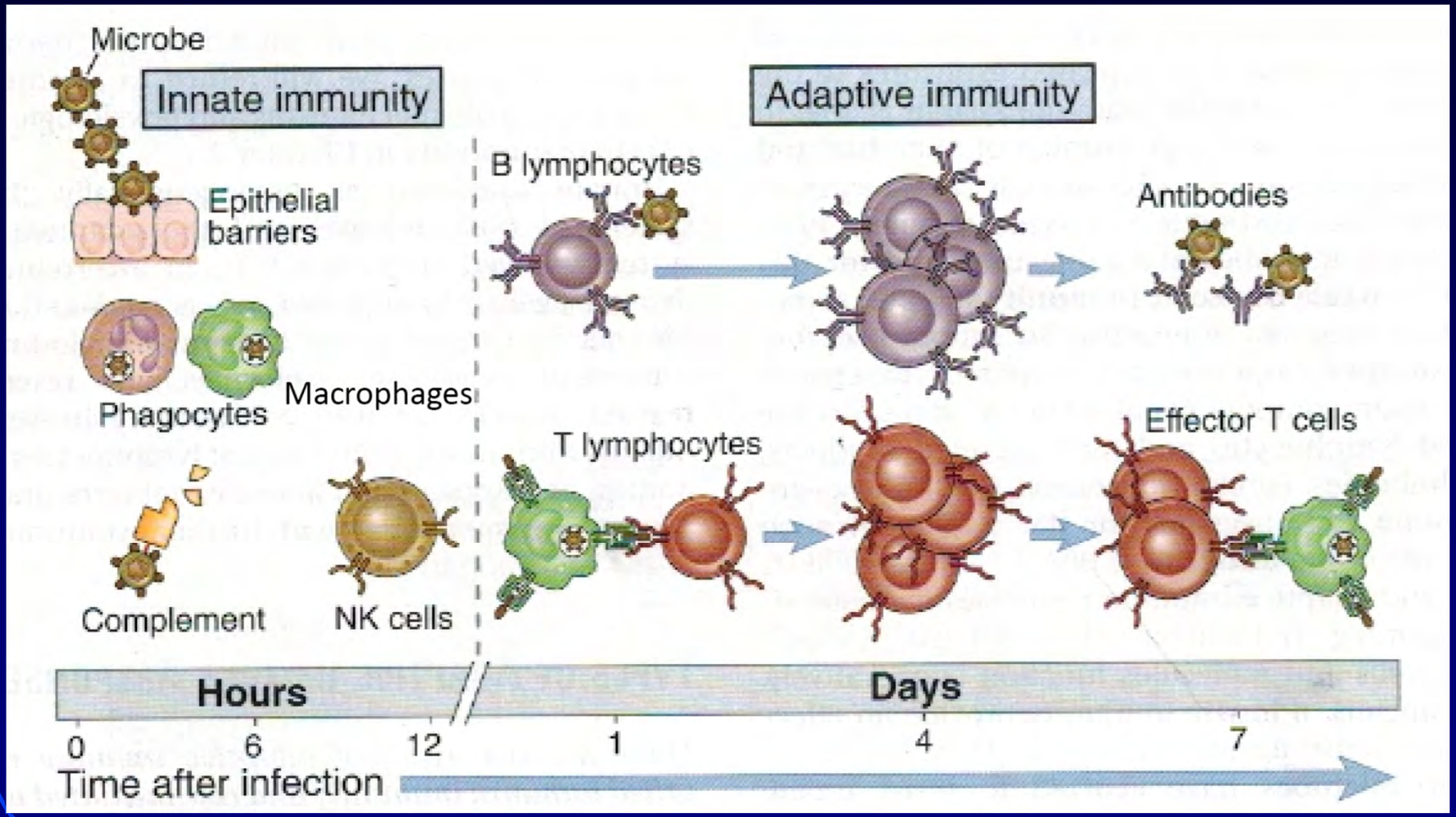
Defining cochlear immune responses and how they are regulated will lead to better therapies for hearing loss

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 signal**s**

Diverse gene expression patterns across and within organs

Inner Ear Resident Macrophages

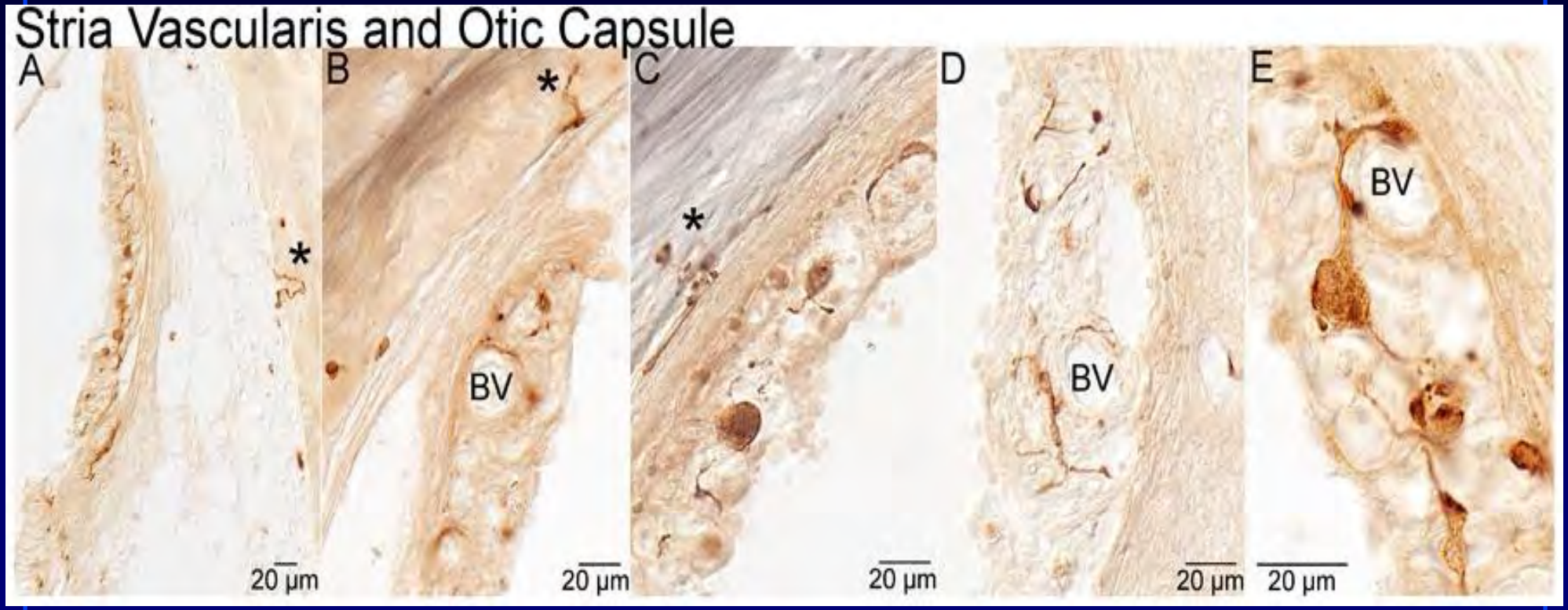
Cochlea: Stria Vascularis

- Spiral ligament
- Scala tympani – periosteum & basilar membrane
- Modiolus – limbus, osseus spiral lamina
- Round window membrane

Vestibular macula, saccule, utricle

Endolymphatic duct and sac

Stria Vascularis



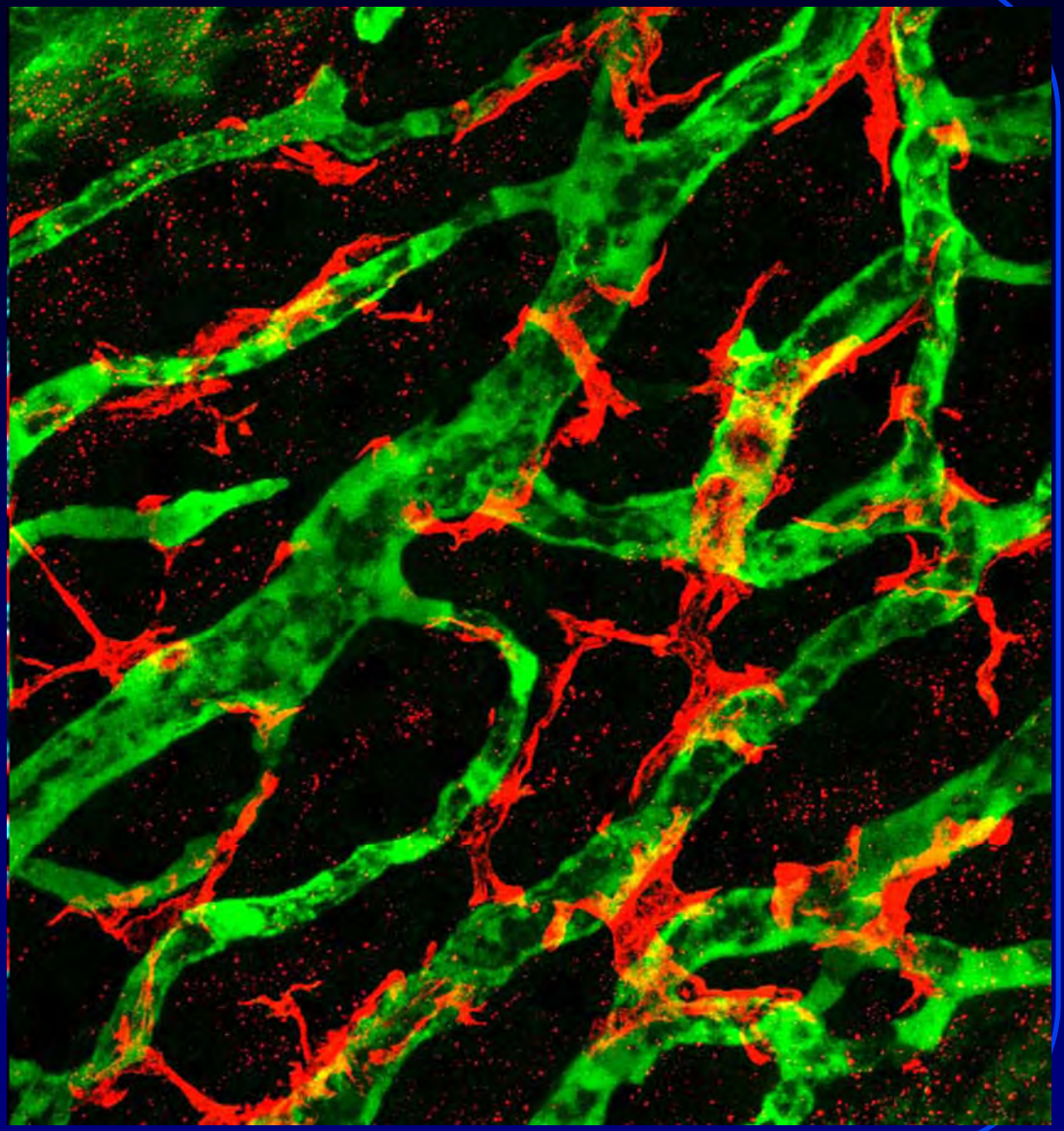
Ionized calcium-binding adaptor molecule 1 (Iba1)-positive macrophages

Jennifer O'Malley and Mike McKenna, MD
MEEI, Massachusetts Eye and Ear Infirmary. Harvard Medical School.

Stria Vascularis

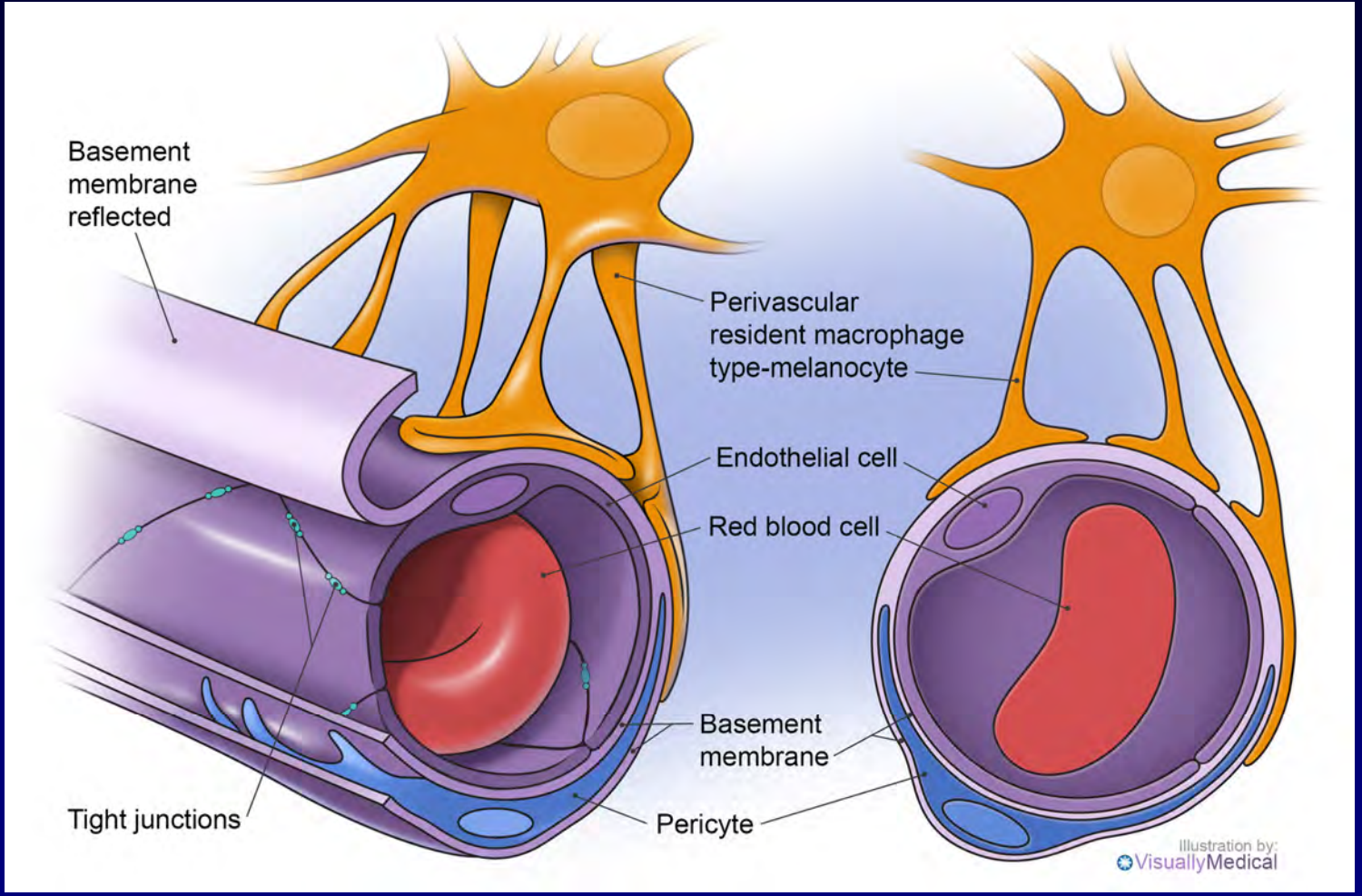
CD31/IBA1

IBA1^{+} macrophages
P14 mouse
whole-mount prep



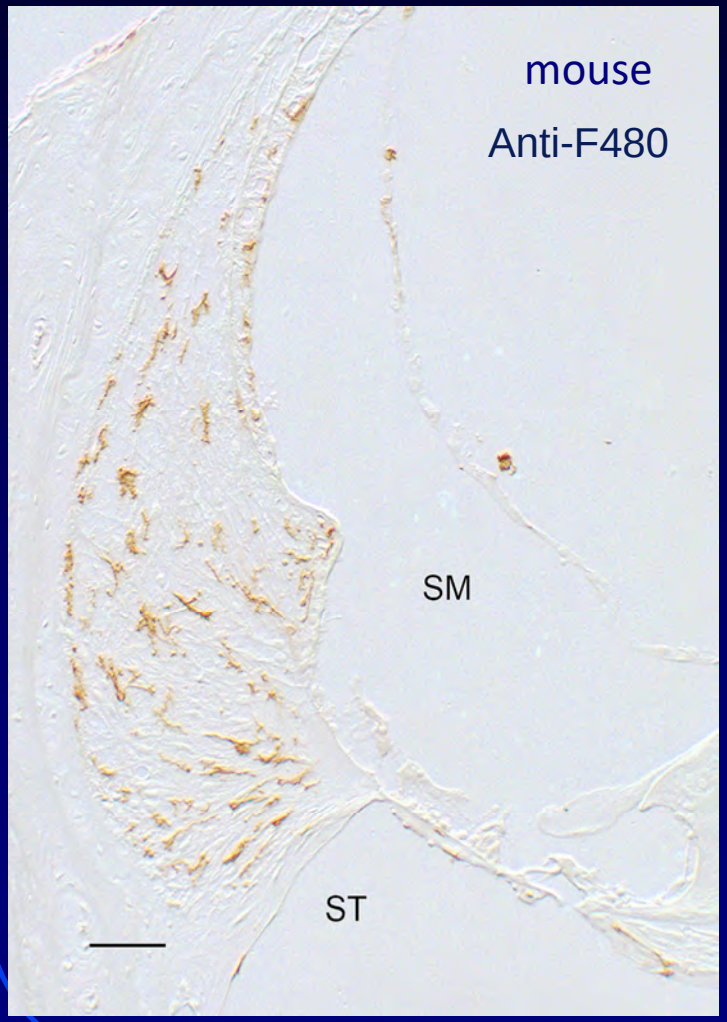
Dr. Hainan Lang, MUSC
(unpublished)

Macrophages in Stria Vascularis

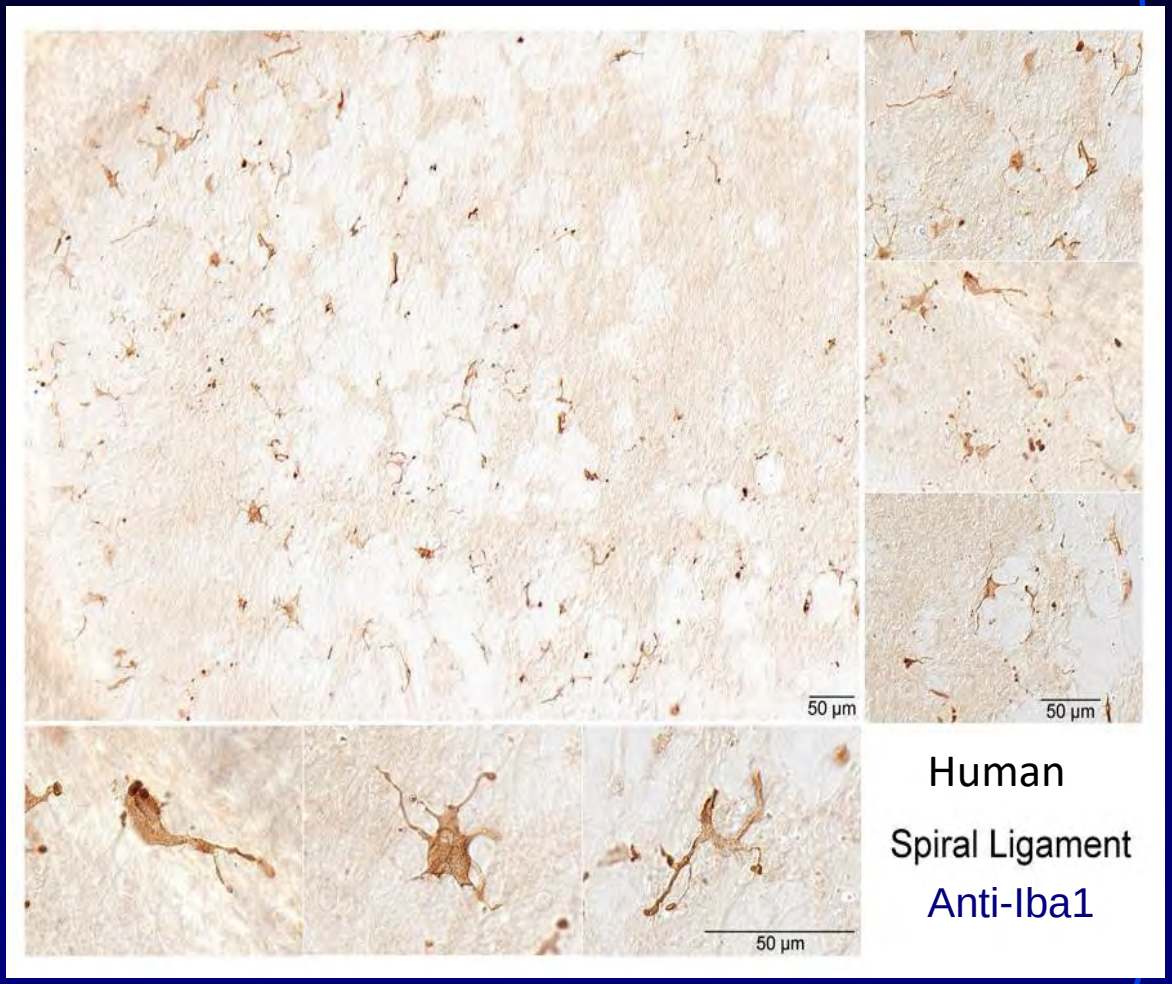


Xiaorui Shi, 2016. Oregon Health and Science University

Spiral Ligament



Masumichi Miyao



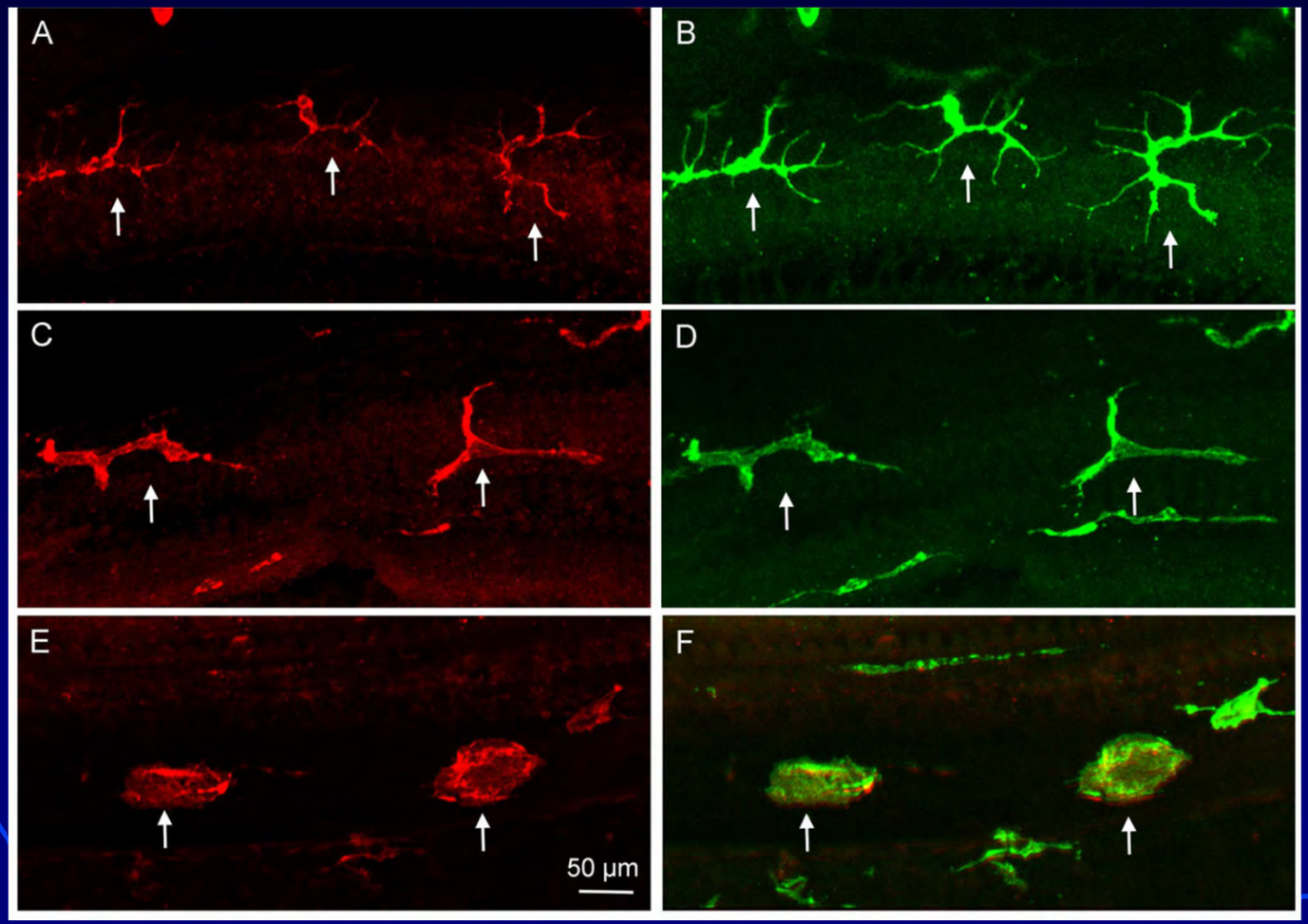
Jennifer O'Malley and Mike McKenna

Basilar Membrane

mouse

Anti-F480

Anti-CD45



apical

middle

basal

Hu, **Zhang**, and **Frye, 2018**

Sensory Epithelium

Jennifer O'Malley and Mike McKenna

Sensory Epithelium

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Macrophages and Hair Cell Degeneration

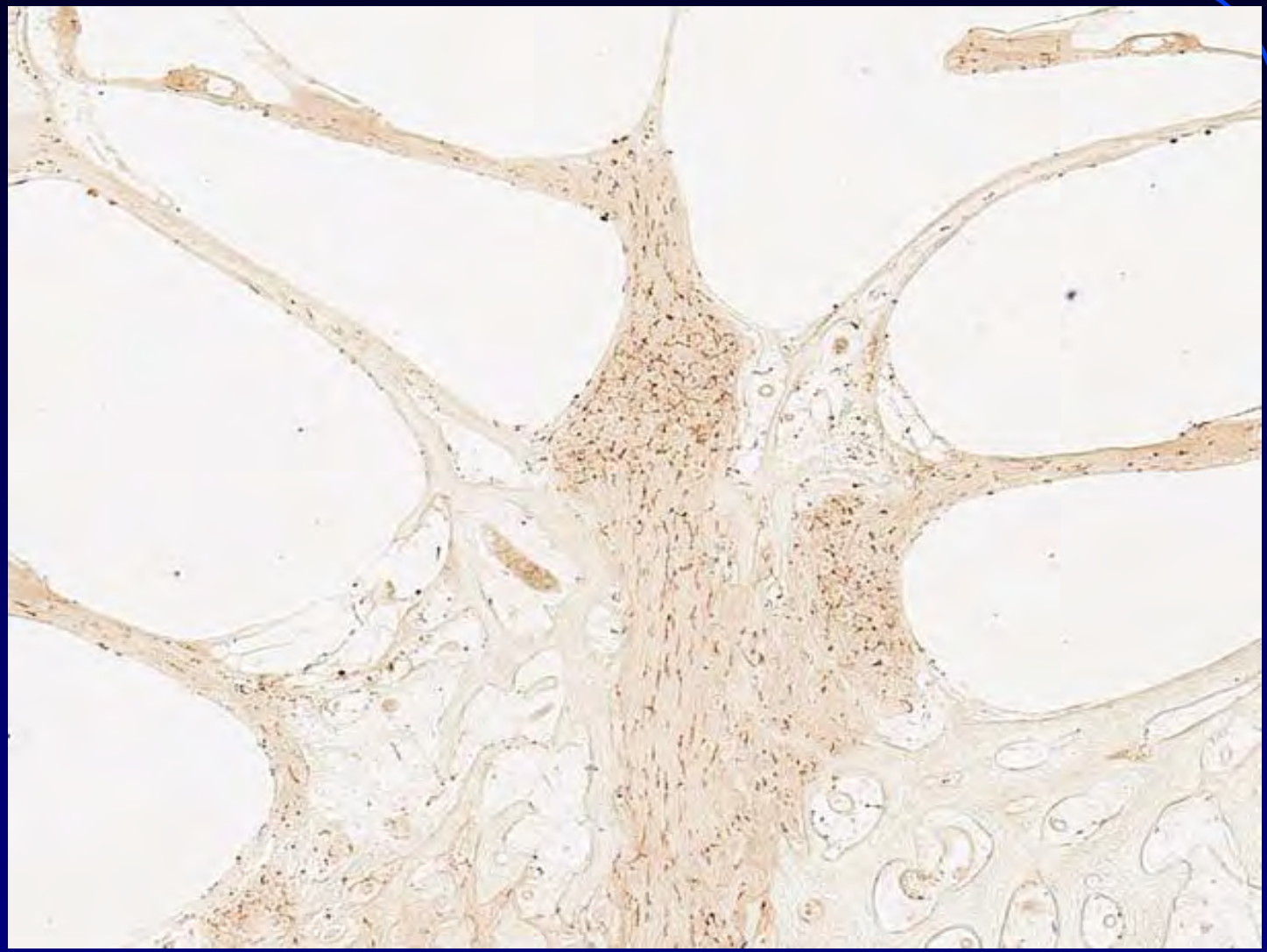
Dying HC are phagocytosed by Deiter's cells.

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

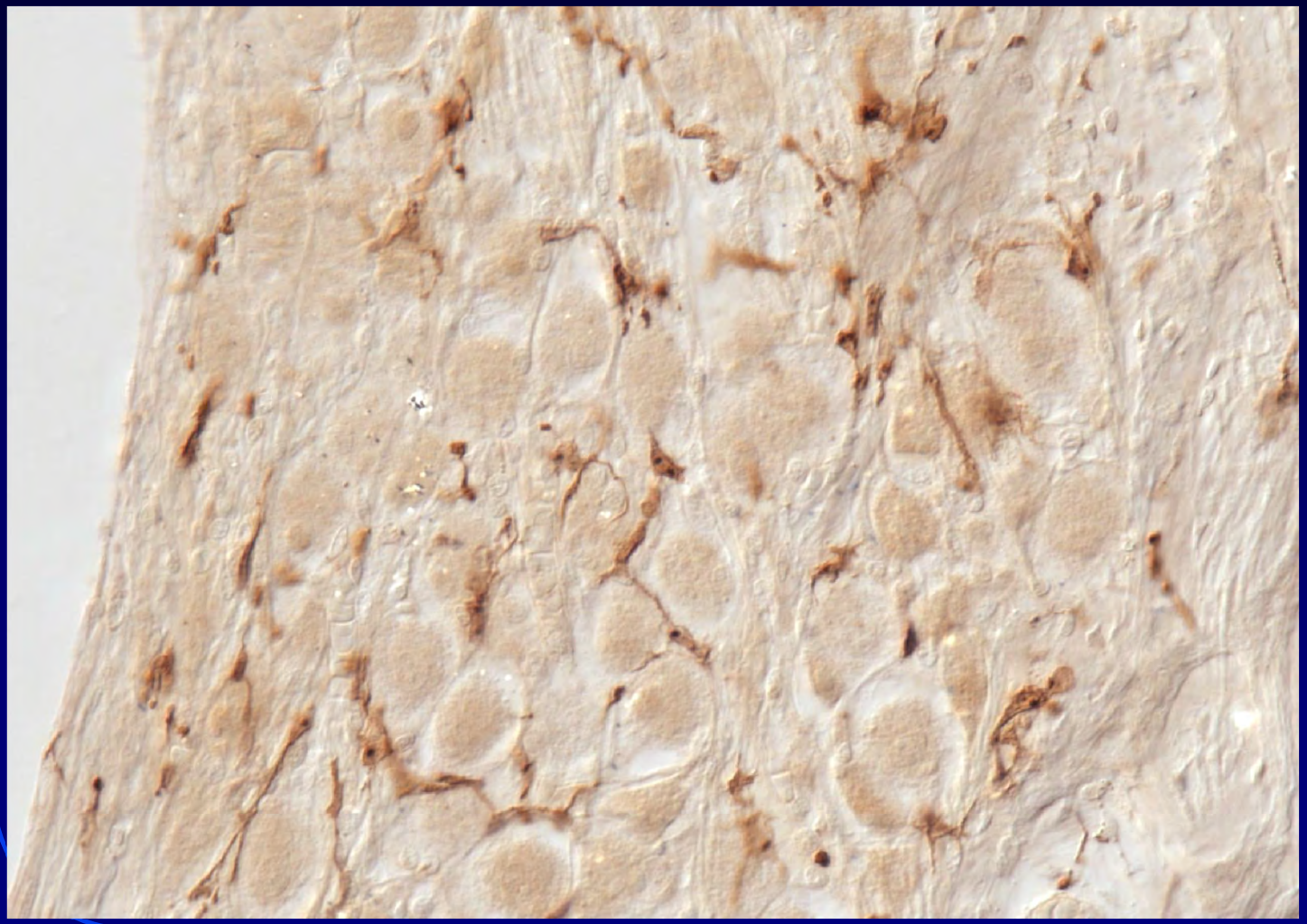
ganglion, nerve, modiolar artery & vein, osseus spiral lamina & limbus



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

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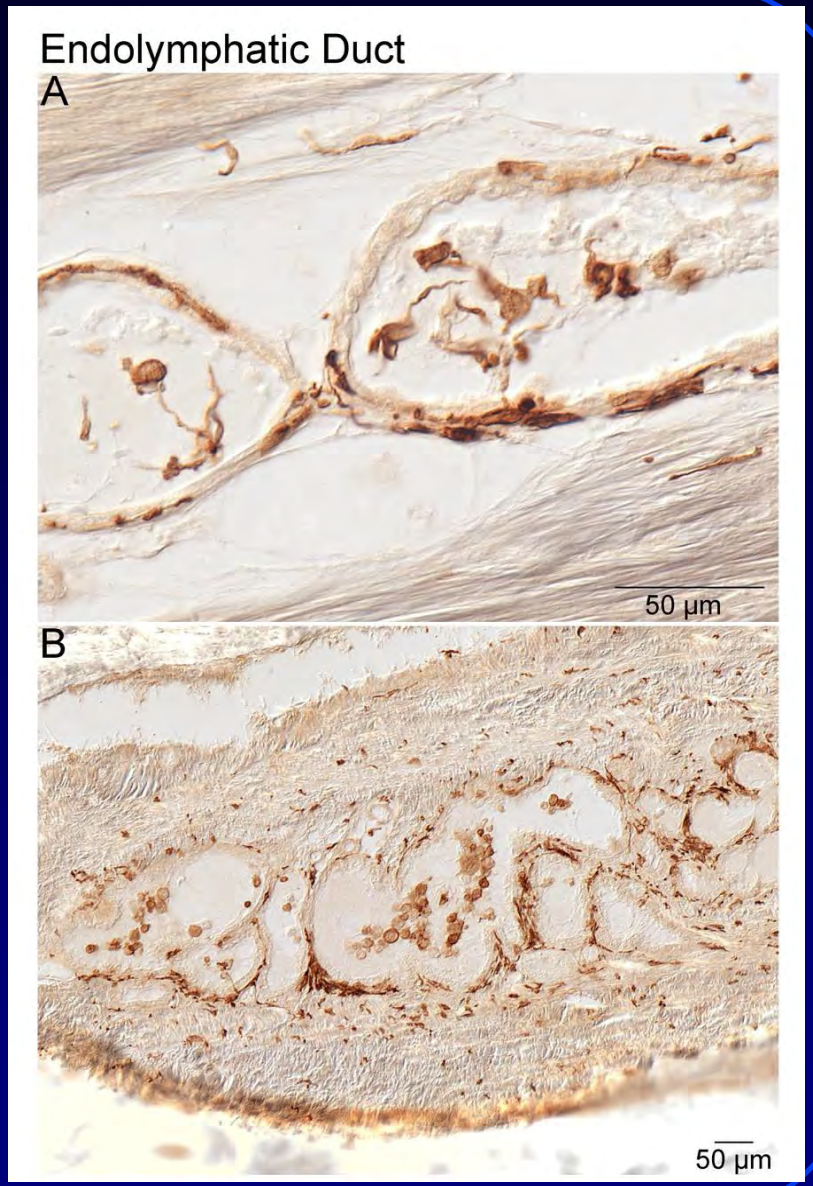
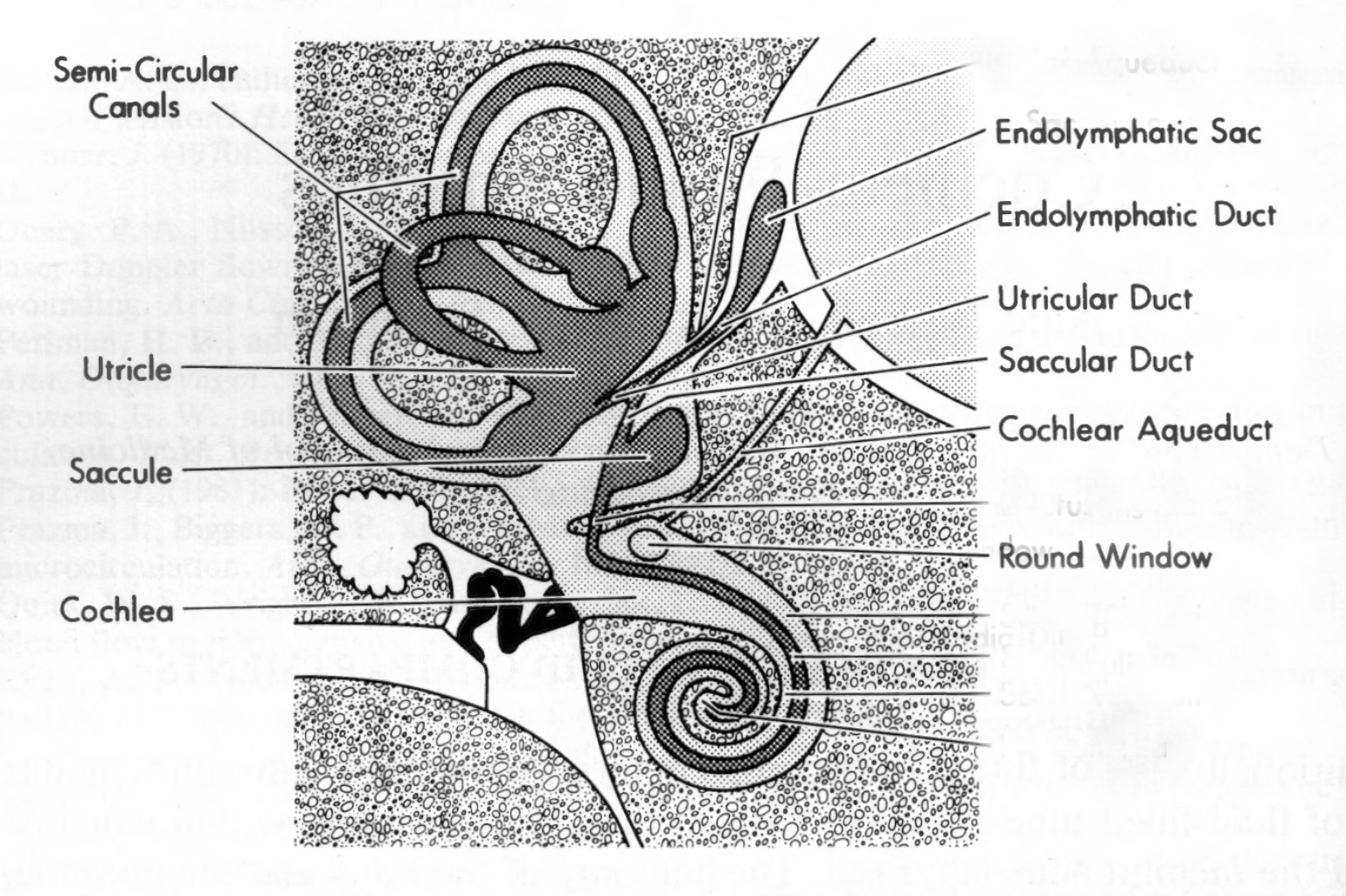


Inner Ear – perilymph and endolymph



Modified from: Jahn & Santos-Sacchi Physiology of the Inner Ear. Raven Press. 1988.

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.

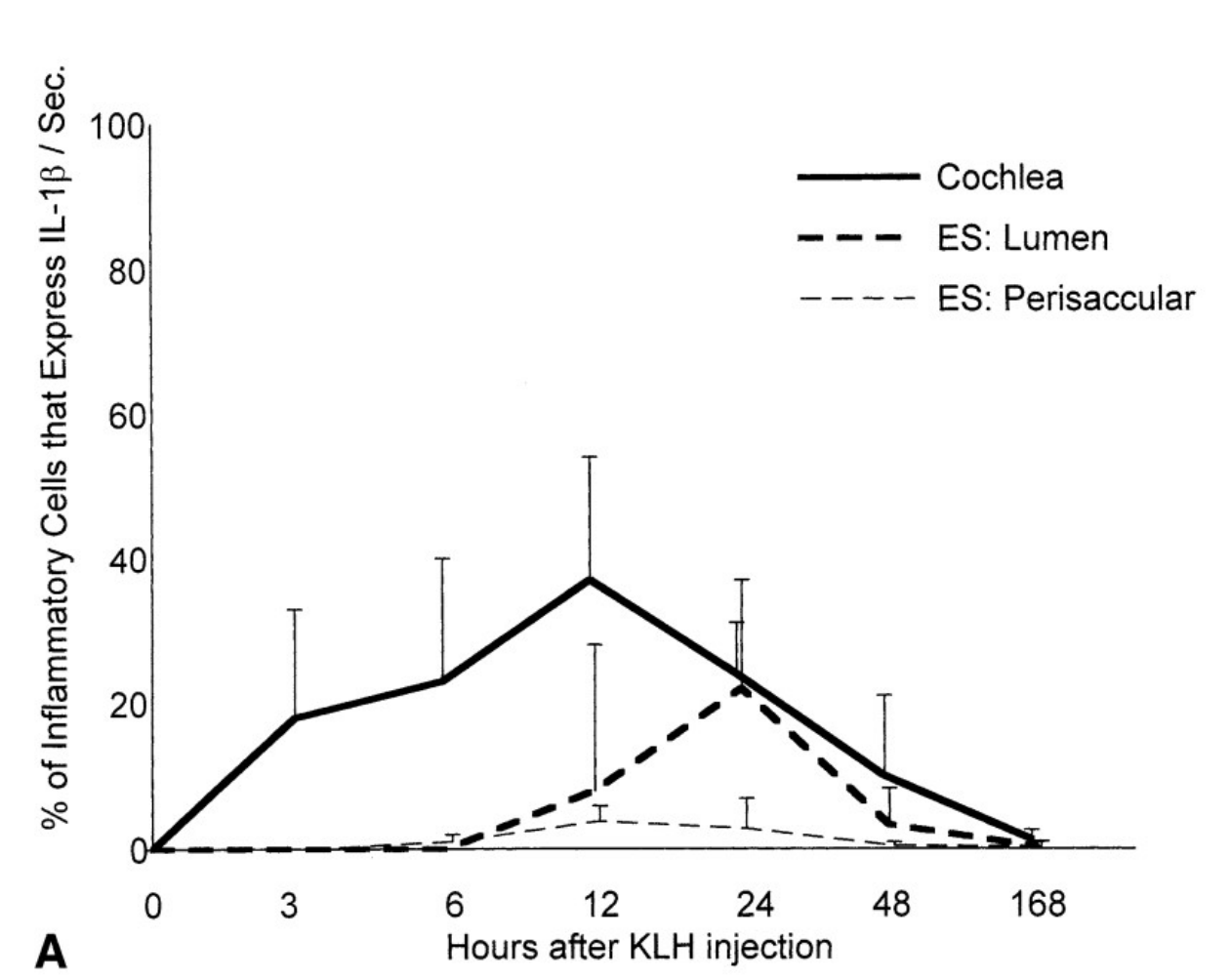
Relationship between Cochlea and Endolymphatic Sac

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

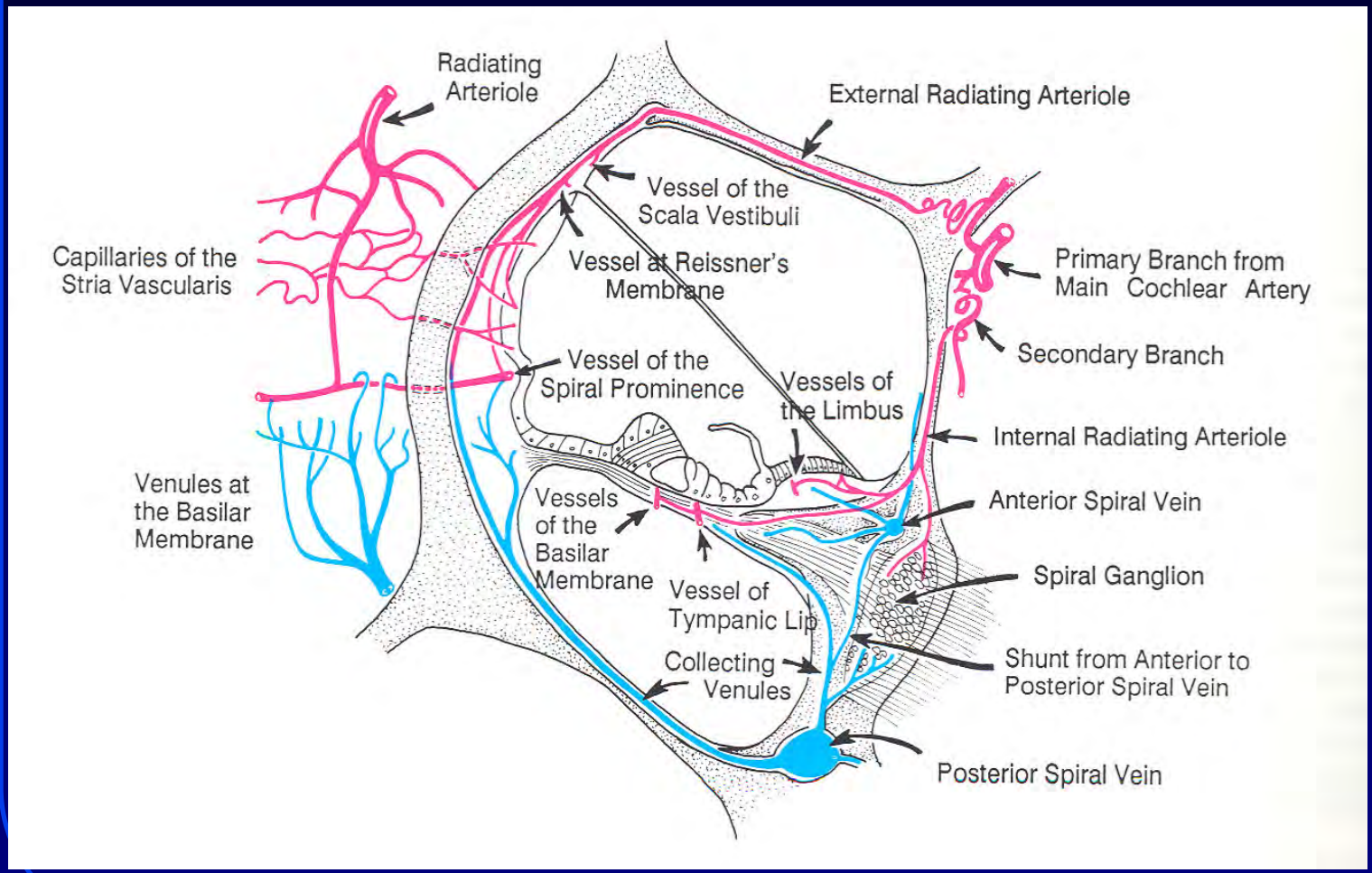
The endolymphatic sac tissue with its vascular, lymphatic and immune cells operates in a coordinated manner with the inner ear immune responses.

Tomiyama and Harris, 1986, 1987, 1989
Satoh et al., 2003

Communication Between the Inner Ear and Systemic Immune System

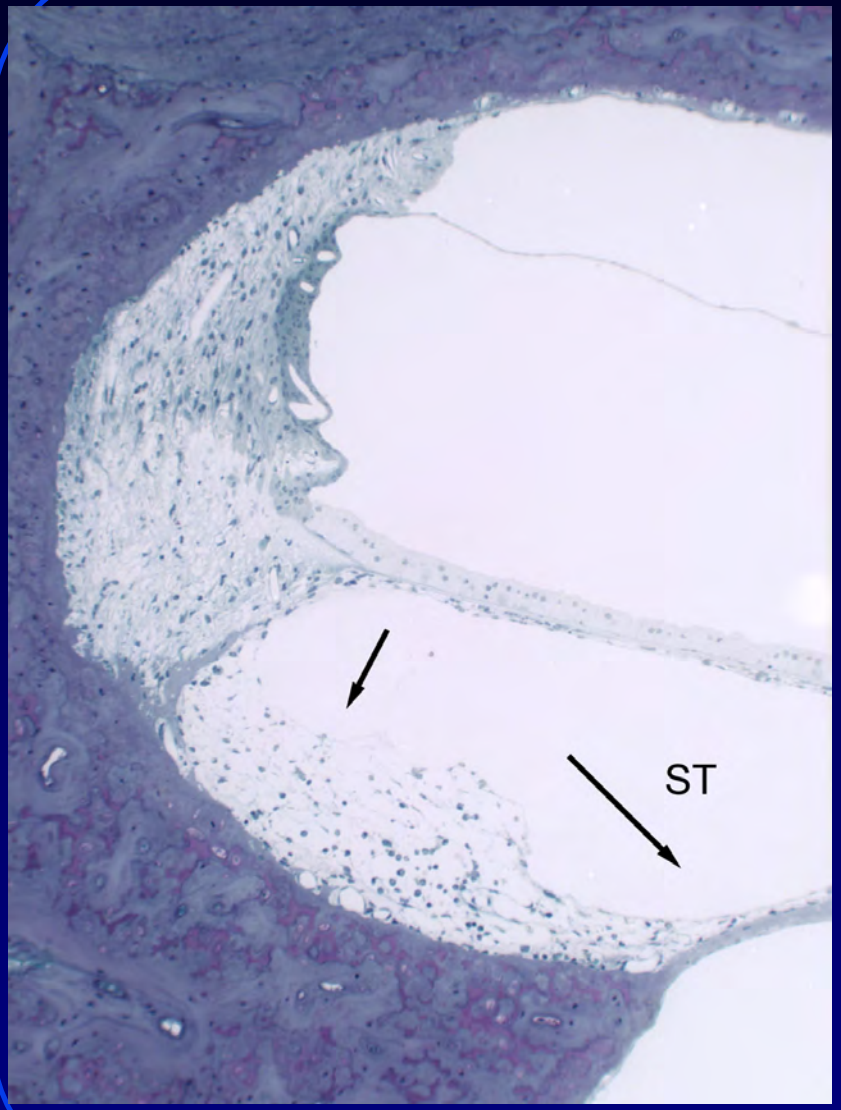
Vasculature / Circulation
Lymphatics

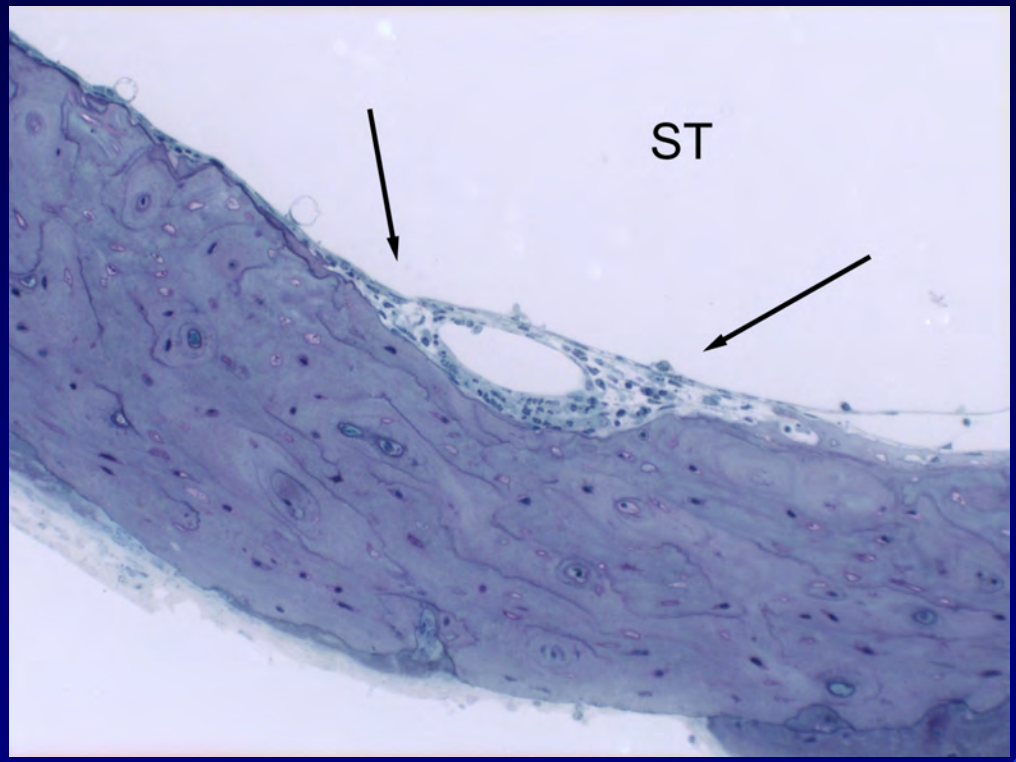
Cochlear Duct Vascular Anatomy



Schuknecht, 1993

Inflammatory cells enter ST from systemic circulation





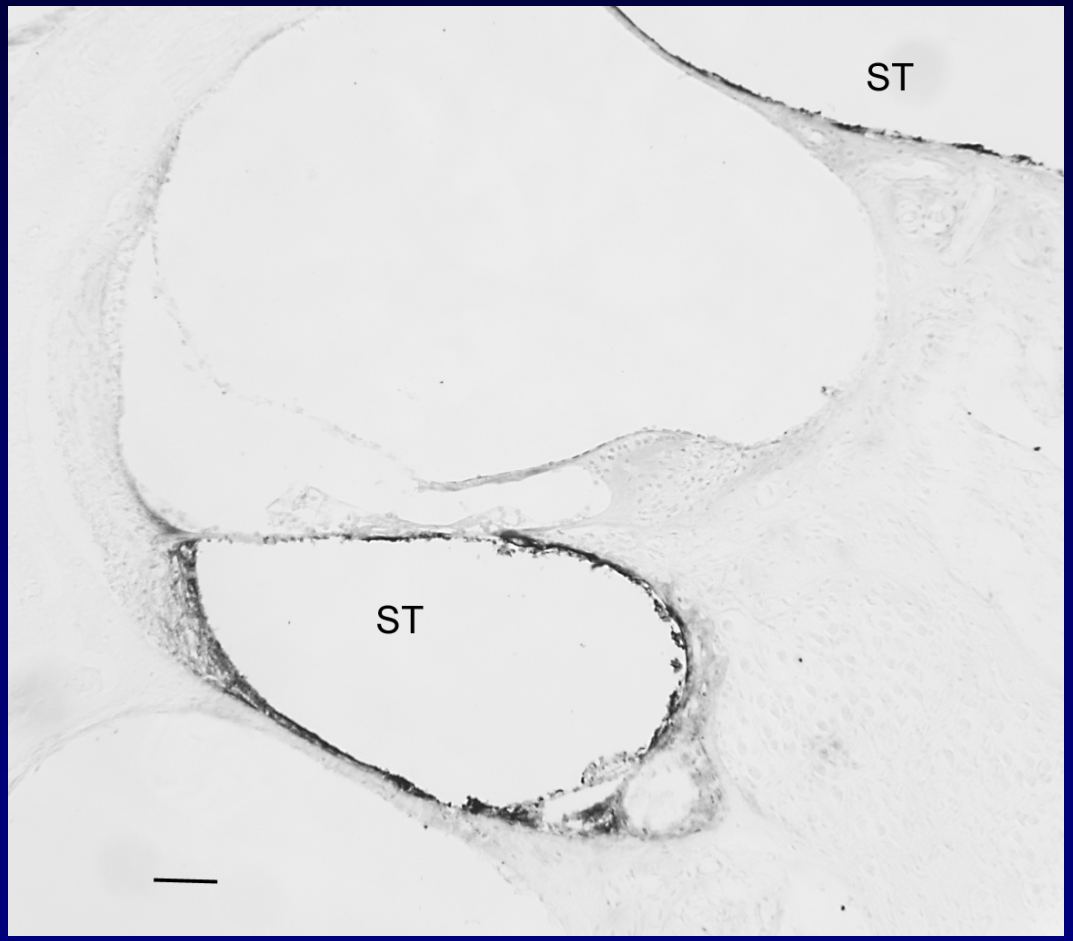
Inflammatory cells enter ST from systemic circulation

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

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

The matrix can become ossified. Always? How regulated?

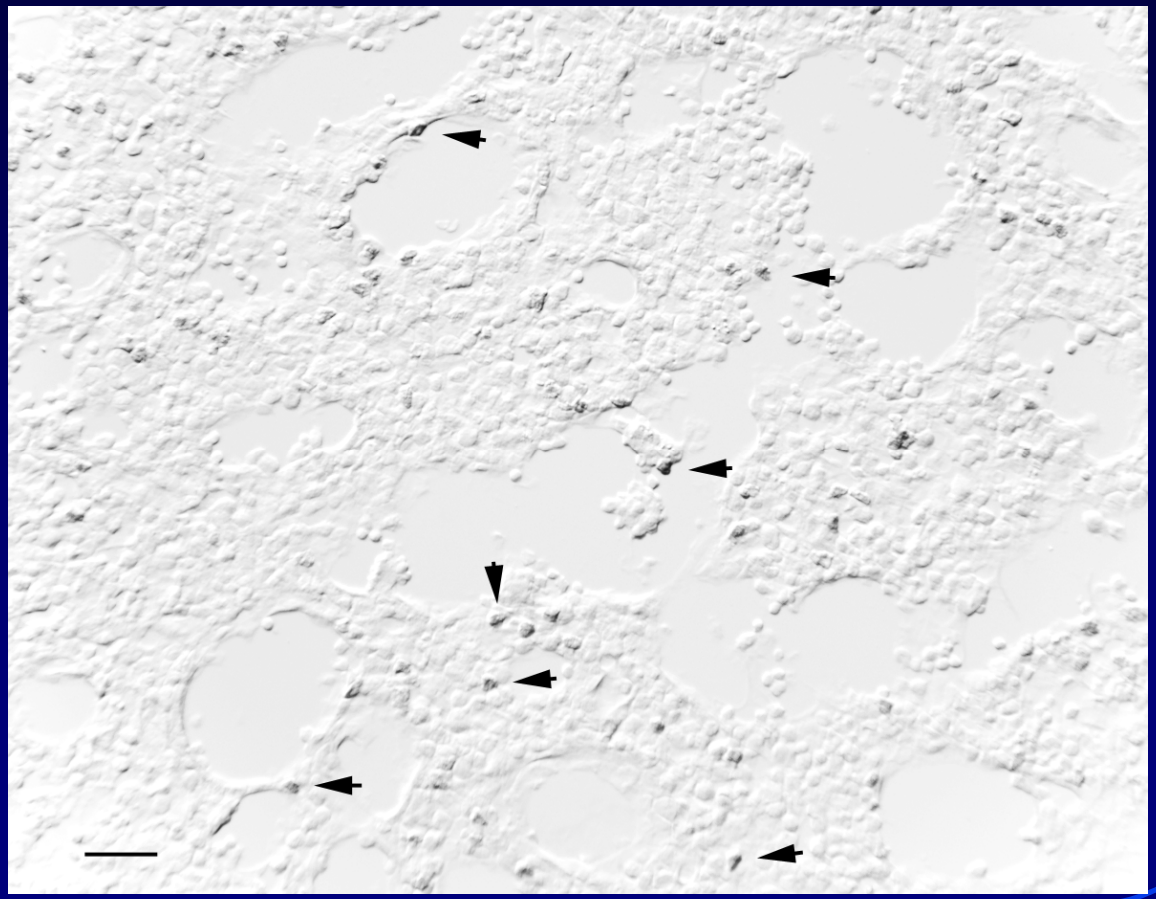
Lymphatic Drainage of Inner Ear



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

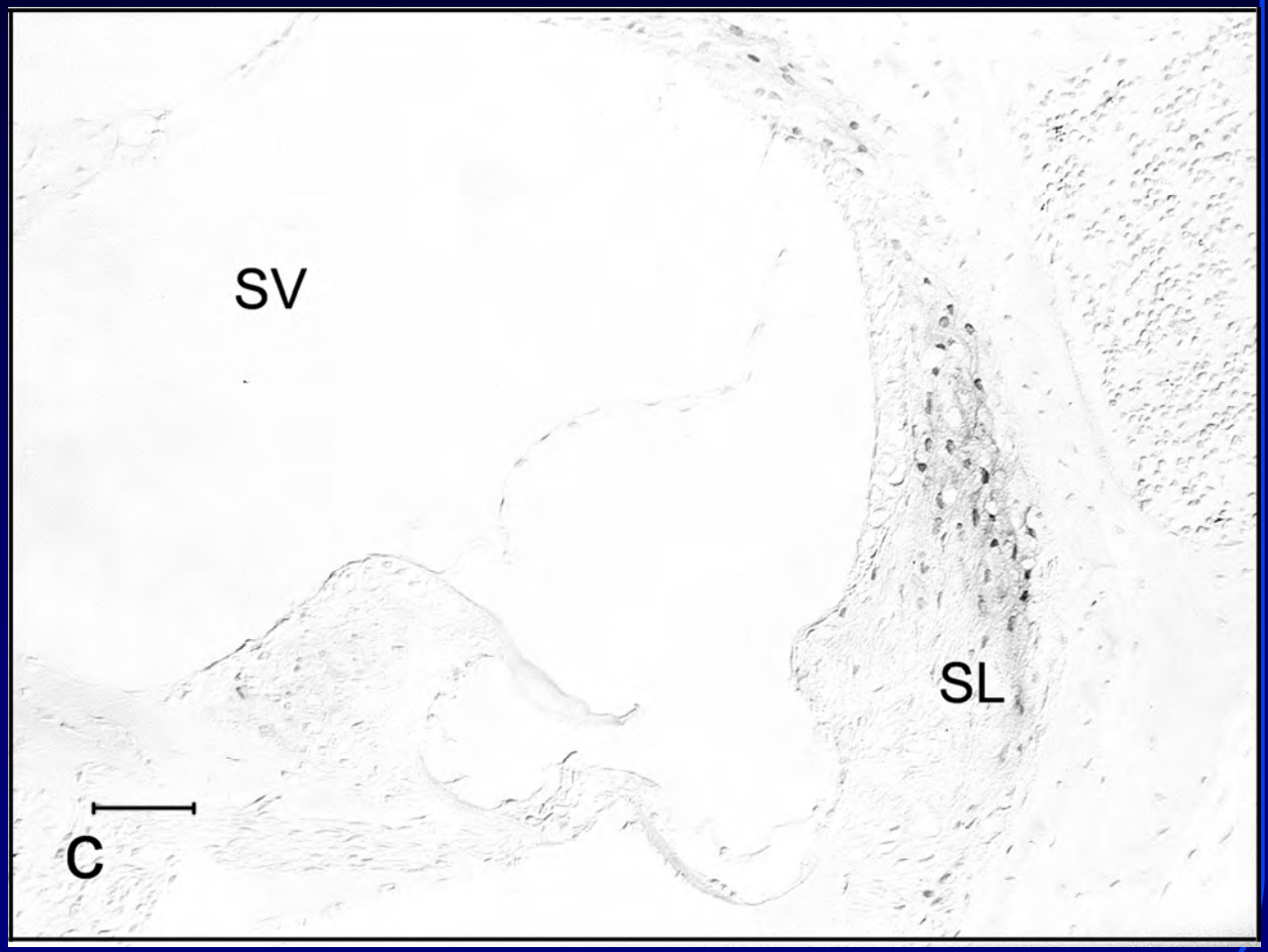
Type I spiral ligament fibrocytes express IL-1β Hashimoto et al., 2005

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

Noise damage is increased – Herranen et al., 2018

IL-1β Expression in Spiral Ligment Fibrocytes

72 hours after i.p.
Injection of LPS

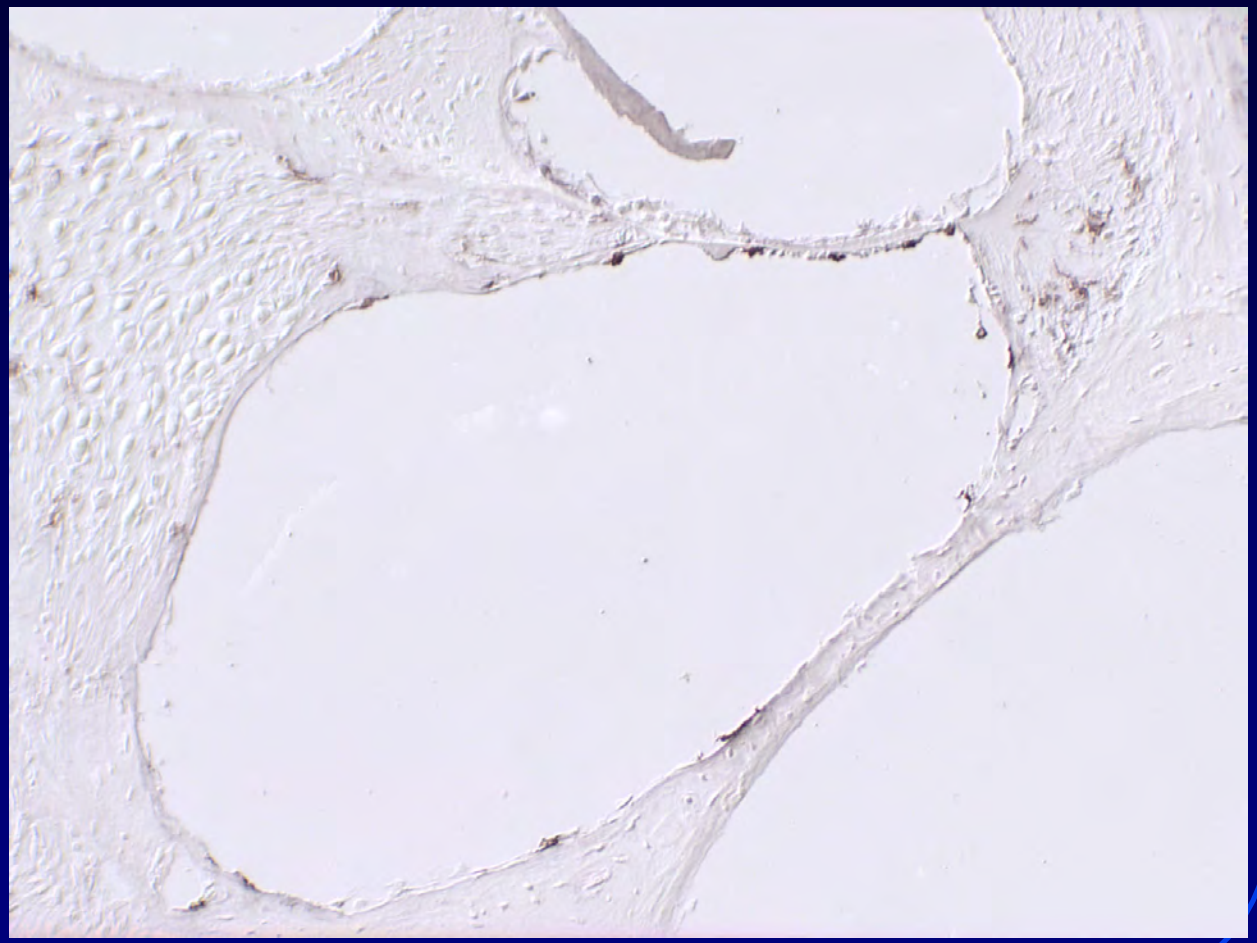


Hashimoto et al., 2005

Cochlear response to Systemic LPS

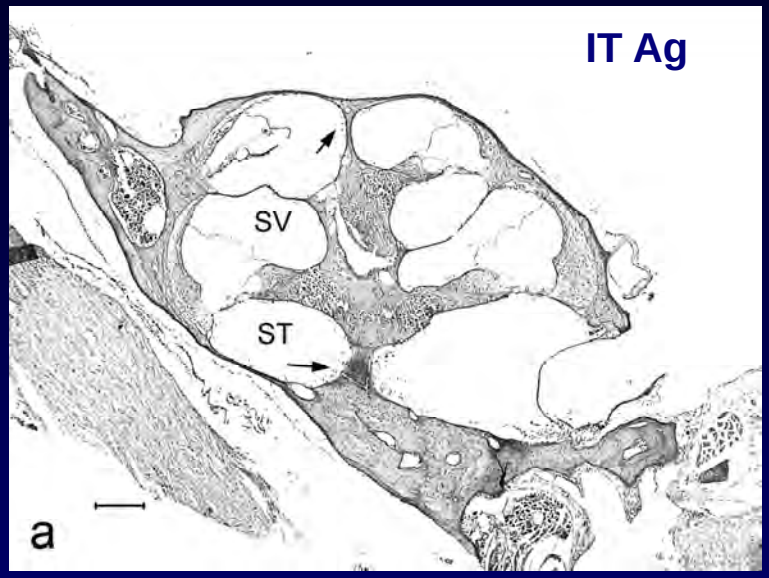
72 hours after
i.p. Injection of LPS

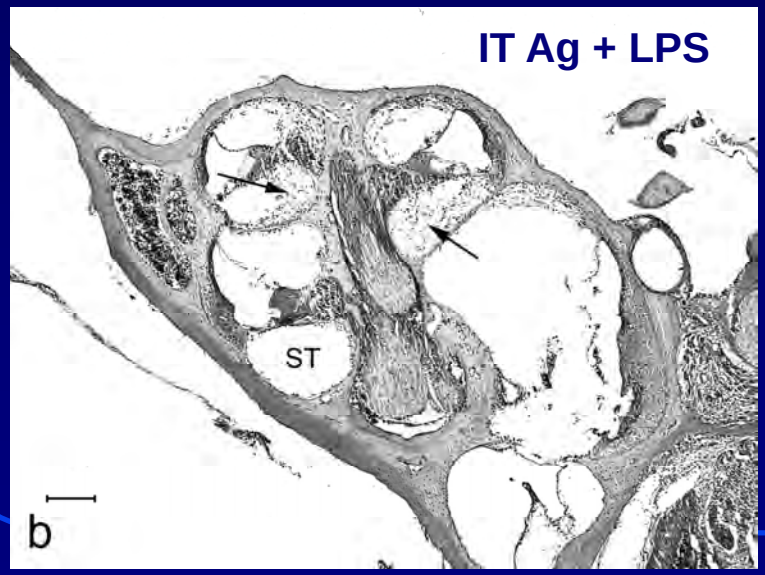
CD-45 immunolabelled – leukocytes in the spiral ligament and scala tympani

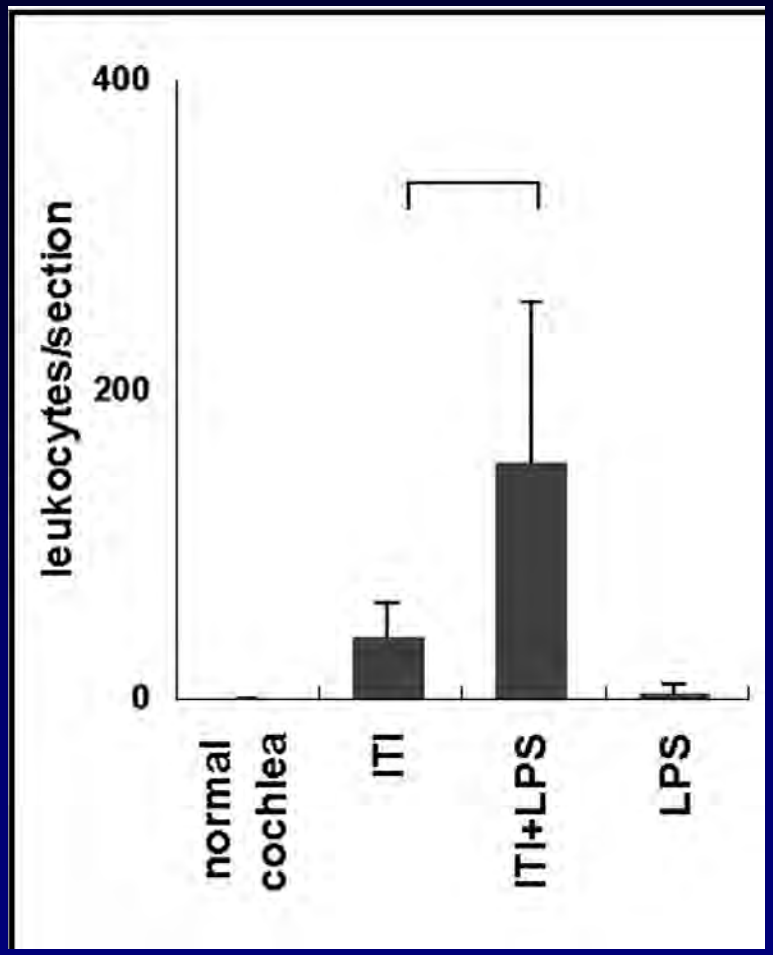


Hashimoto et al., 2005

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

Immune Response to Acoustic Trauma

Macrophages assist in repair of damaged HC-SGC synapses (Manickam et al., 2023).

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

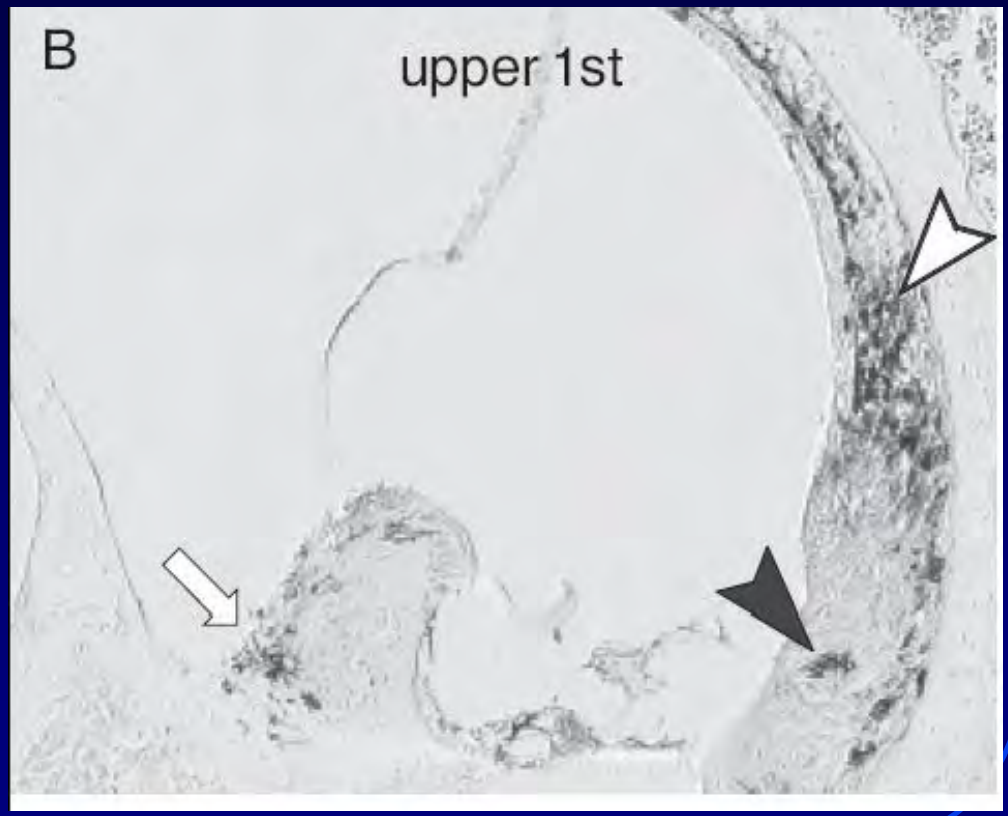
Secretion of pro-inflammatory
cytokines such as: TNF-α, IL-1β and IL-6 and
chemokines such as: MCP-1, MCP-5, CCL2

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

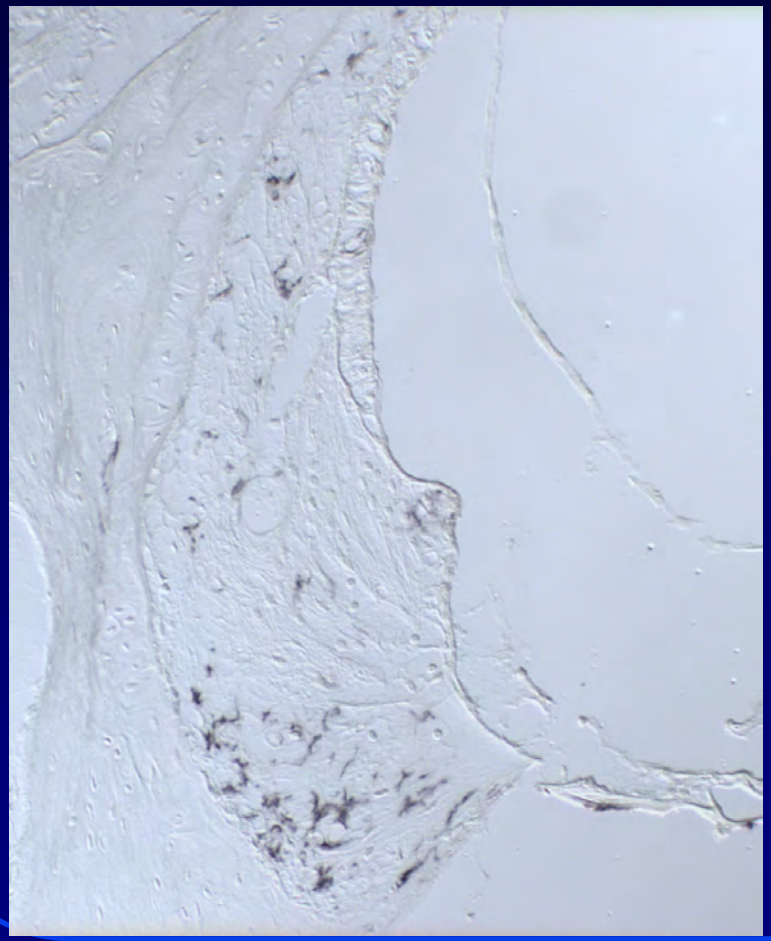
Activation of NF-κB, a transcription factor for cytokines, in type I spiral ligament fibrocytes.



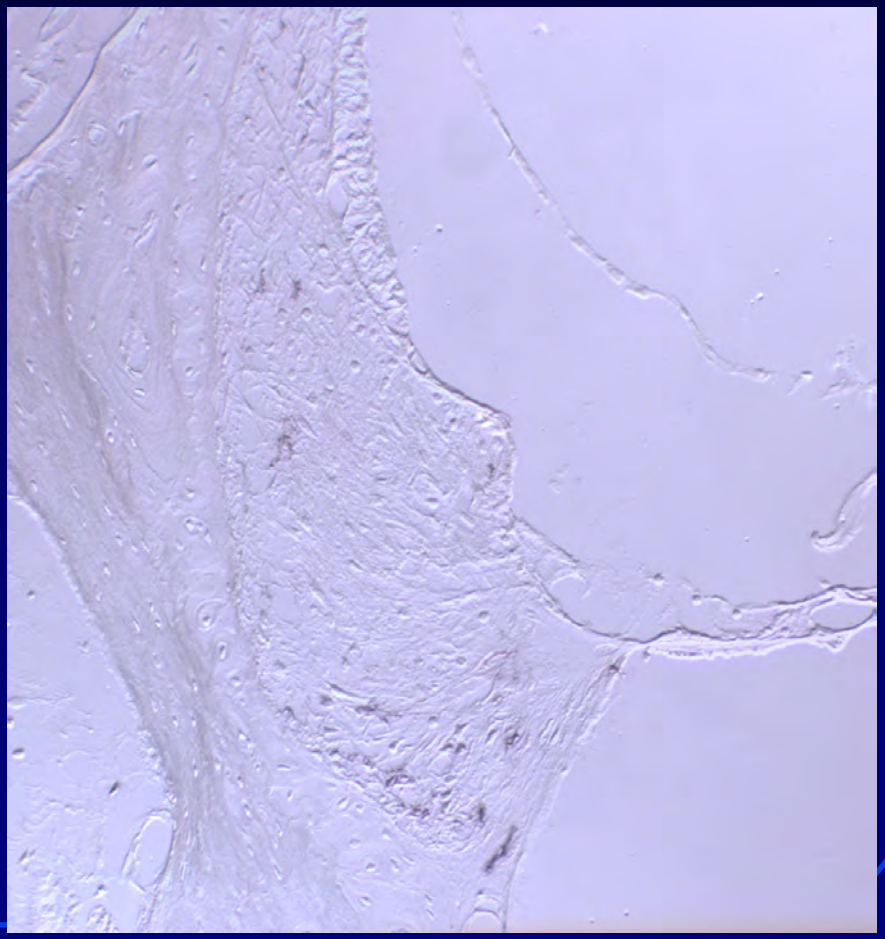
Adams et al., 2009

Macrophages in Spiral Ligament - acoustic trauma

F4/80 – activated macrophages

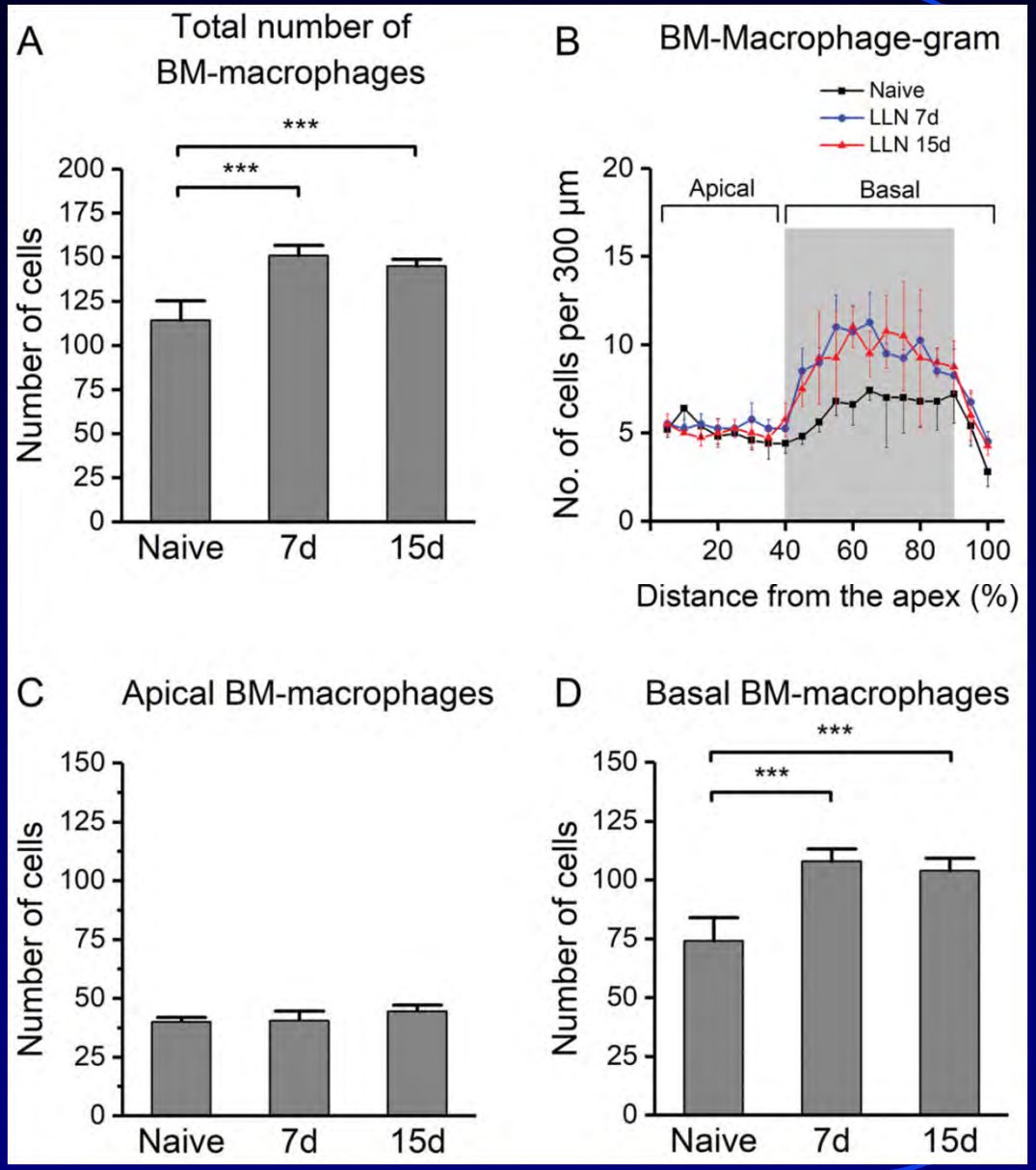


CD-45 pan-leukocyte



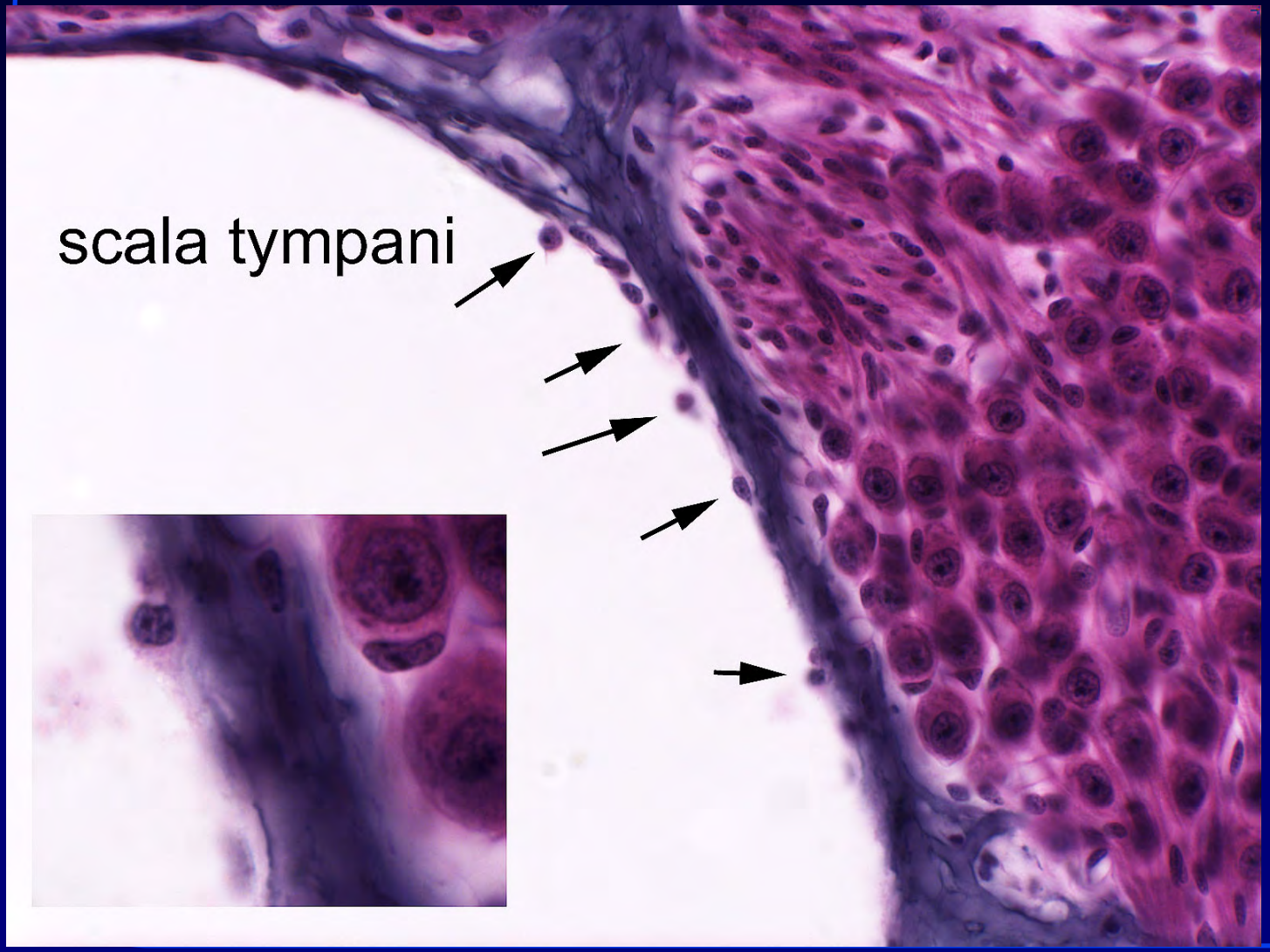
Basilar Membrane

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



Frye, Zhang & Hu, 2018

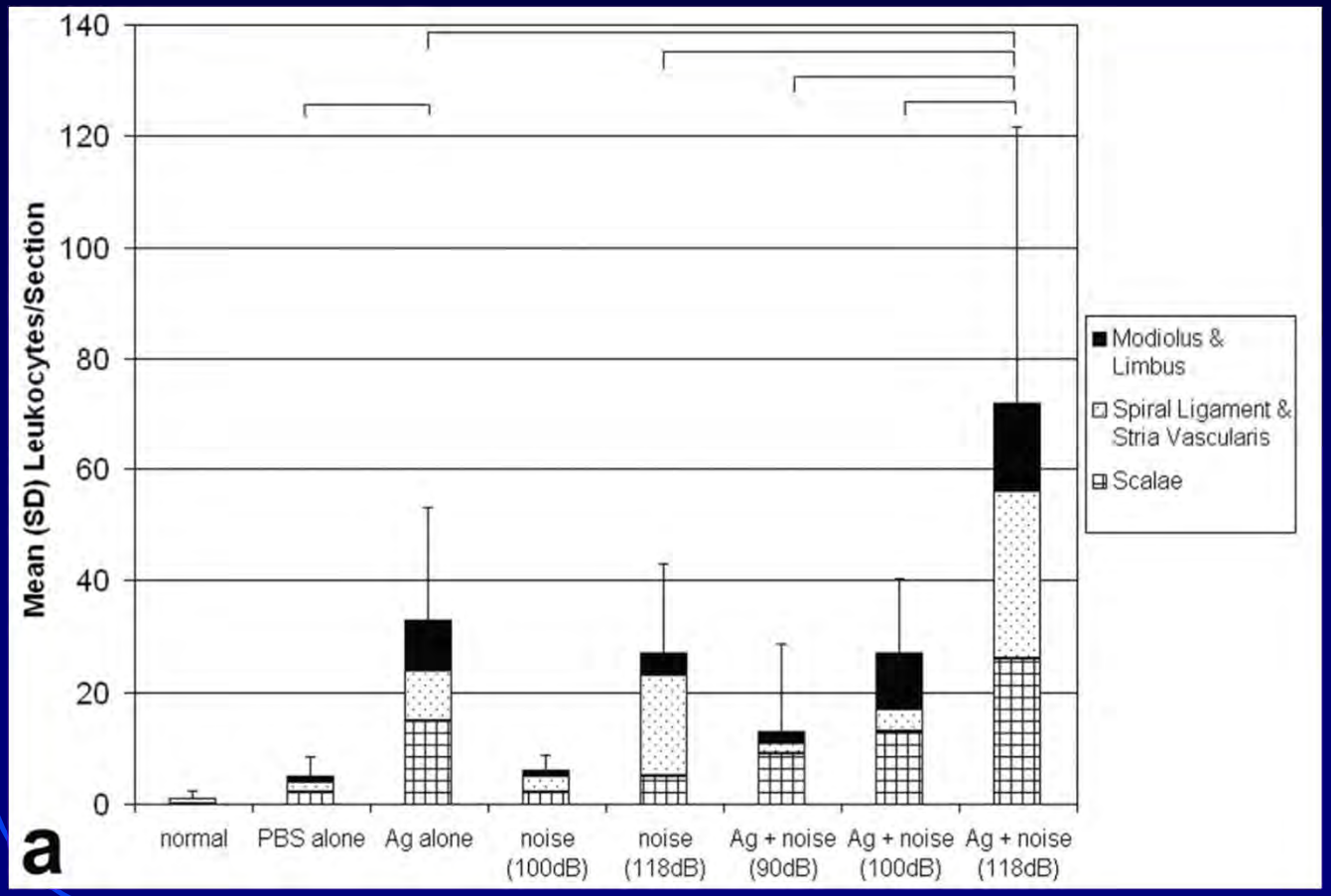
Infiltration of Leukocytes after Acoustic Trauma



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

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