IMMUNOLOGIC SENSORINEURAL HEARING LOSS
: AN EVIDENCE OF ANIMAL STUDY

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Abstract Experimental studies and clinical evidence suggests immune-mediated induced sensorineural hearing loss have been investigated in an animal model (guinea pig and mice), produced by immunization with inner ear tissues (chick and guinea pig cochlea). ABR testing revealed significant hearing loss in seven of eight guinea pigs immunized with chick cochlea. With ELISA methods, serum from all animals immunized with cochlear antigen developed antibodies to cochlear antigens of both chick and guinea pig inner ear tissue. Application of immunoperoxidase techniques revealed immunostaining of hair cell stereocilia using serum from guinea pigs immunized with chick inner ear tissues. Histologic examination was similar to endolymphatic hydrops. Cross-species immunization producing a humoral response associated with significant hearing loss suggests that the immunologic etiology may play role in the pathogenesis of idiopathic sensorineural hearing loss. Chiang Mai Med Bull 1992; 31: 183-192.

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Introduction

Acute idiopathic sensorineural hearing loss (SNHL) affects 1 : 10,000 individuals per year,(1) and immune-mediated disease may be one of the pathogenesis in such hearing disorder. Several mechanisms have been proposed that could precipitate the autoimmune ear disease, such as the release of sequestered inner ear antigen by trauma or infection. The expression of embryonic cartilage antigen (type II collagen) in the otic capsule may produce an foci of
otosclerosis. Infectious agent or chemicals could alter their surface proteins such that they are no longer recognized as autologous, similar to the process which occurs in rheumatic heart disease. Genetic factors may also be involved as Cw7 HLA haplotype conferred a greater susceptibility to develop hearing disorder.

Experimental and clinical studies over the last decade have suggested that autoimmune disease can involve the inner ear by collagen-vascular pathogenesis. Foci of vasculitis with lymphocytic infiltration and granulomatous inflammation have been found in patient with Cogan’s syndrome, Wegener’s granulomatosis, relapsing polychondritis, and rheumatoid arthritis.

In vitro studies using lymphocyte proliferation/migration inhibition techniques, and RIA-CII antibody assays, have demonstrated serologic evidence of an activated immune response in patient with Cogan’s syndrome, autoimmune SNHL, otosclerosis and Meniere’s disease, when compared to sera of normal control subjects. A guinea pig model of autoimmune SNHL has also been proposed by Harris. In animal immunized with bovine cochlear tissue (CCAg), 12 of 38 ears experienced significant loss of hearing in association with elevated titers of endogenous cochlear antibody measured in the serum and perilymph.

This study was under taken to investigate an animal model of immune-mediated hearing loss produced by cross-immunization with inner ear tissues. Experiments using appropriate controls were designed to examine the following response:

1. Determine if hearing dysfunction occurs in immunized subjects by cross-immunization.

2. Determine if there is production of antibodies to endogenous cochlear tissue by ELISA.

3. Identify the site of antibody binding to cochlear neuroepithelium by immunocytochemistry.

4. Determine if there is histologic evidence of inner ear pathology by histopathological section.

Materials and Methods

The experiment was done at the Kresge Hearing Research, Department of Otolaryngology-Head and Neck Surgery, University of Michigan, USA. at the period of February 1, 1989 - July 31, 1990.

Animals

Subjects studies included ten balb/C mice and ten NIH-strain pigmented guinea pigs weighing between 150-250 g. Animals in each group were controlled for age and weight, and fed a standard rodent chow diet and water.

Antigens

Cochlear duct tissue was harvested from
two-day old chick hatchlings using a microdissection technique described by Raphael. (13) Tissue specimens were pooled in physiologic saline and homogenized to a concentration of 2,000 µg protein/500 µl. Inner ear tissues obtained from 200 gm guinea pigs subjects, 1,000 µg/500 µl, included the cochlear and modiolar portion of the acoustic nerve. Specimens from both species were performed in a bath of phosphate-buffured saline solution, pH 7.4.

1. Immunization

Prior to immunization, a cardiac puncture was used to collect 1.0 mL of blood from guinea pigs, and 0.33 mL from orbital vein puncture of the mice. (14)

In the 10 guinea pigs, eight animals underwent inoculation with a chick cochlear tissue, two animals as control were injected by normal saline. Two subsequent boosts of injection (chick cochlear tissue or normal saline), at the identical concentration, were given at two and four weeks later in each group (three injections totally).

In the 10 mice, four received chick cochlear tissue, four received guinea pig cochlear tissue, and two received normal saline as control. Three subsequent boosts of injection in the three groups were given at two, five and ten weeks later.

All immunizations were administered by intraperitoneal and subcutaneous injection. At the conclusion of the five weeks immunization for the guinea pig group, and the five and ten weeks for the mouse group; all animals underwent a terminal bleed for post-immunization serum prior to sacrifice.

Hearing test

The hearing of each animal was assessed by threshold measurement using far-field ABR recording under sedation with Ketamine at 0.6 µl/gm BW. The ABR equipment consisted of a Hewlett-Packard sound generator model 302 A and a Wilsonics tone switch. The following recording was used: tone burst of 2,615 kHz delivered at 3/sec, 15 ms duration, rise time of 5 ms, and stimulus intensity of 96 dB SPL (15 kHz), and 103 dB SPL (2 kHz).

2. ELISA

Antibody levels to cochlear antigens were measured utilizing an avidin-biotin conjugated alkaline phosphatase ELISA technique. Coated microtiter plates with chick and guinea pig cochlear homogenate were incubated for one hour at 37 °C with serial dilutions (log 5) of pre and post immunization sera. Non-specific binding sites were blocked with 300 µl of phosphate buffered solution (PBS) containing 1% normal goat/horse serum for 30 minutes. After washing with PBS, 50 µl of avidin-biotin alkaline phosphatase reagent as ligand was added and incubated for 30 minutes at 37 °C. A hundred µl of p-nitrophenylphosphate (as substrate) was added and allowed to incubate for 5-10 minutes.
at 37 °C. The reaction was stopped by 5 N NaOH, and absorbance reading was measured on a Micro ELISA autoreader.

ELISA results were analyzed by comparing the mean absorbance value (function of protein concentration) at each dilution concentration of immunized and control animal sera. One standard deviation was used to denote the range of variability at each data point.

3. Immunocytochemistry

To detect the immunoreaction between nonimmunized guinea pigs cochlea and post-immunization sera. The non immunized guinea pig inner ear was prepared by cryosection. Then, the cryosections were overnight incubated with pre and post-immunization sera at 4 °C. After washing specimens with phosphate buffered solution, the presence of bound antibody to neuroepithelium was detected using immunofluorescence and immunoperoxidase techniques.

4. Histology

Temporal bones of immunized animals were obtained following open cardiac perfusion of 2 % paraformaldehyde in phosphate buffer. Using the dissecting under microscope and bone drilling, the otic capsule was infused via the oval and round windows with 4 % EDTA, and allowed to decalcify for a period of 10-14 days at 4 °C. Six to eight μm thick section were cut and permanently mounted on glass slides.

Results

During the course of the experiment, all animals tolerated immunization and blood drawing techniques without manifestation of systemic illness and ear diseases.

Hearing loss

SNHL of 10-20 dB was presented in seven of the eight guinea pigs immunized with chick cochlear tissue. Three had bilateral involvements, and four had unilateral loss. No significant change in hearing threshold for the control group was observed. It was found that the threshold at 2 and 6 kHz had returned to preimmunization level at the third week. However, seven days after the third immunization (the fourth week), ABR testing revealed an elevation in threshold at these frequencies of 30 and 35 dB.

The change in latency and magnitude of the P2 wave at 15 kHz is associated with 18 dB increase threshold level for the immunized group (threshold remains constant in the control group). Guinea pigs immunized with chick cochlea manifested increased latency response times associated with a decrease in slope velocity and magnitude of the wave amplitude (Fig. 1,2).

In the mouse group, the presence and degree of hearing loss depended on the type of cochlear antigen used for immunization. The four mice immunized with chick cochlear tissue had bilateral elevations in hearing threshold of 15 to 40 dB at the three and five week test periods.
The mice immunized with guinea pig cochlear tissue and the control animal injected with normal saline did not have significant change in hearing threshold.

**ELISA**

The antibody binding activity between wells coated with chick cochlear tissue and all antisera obtained from guinea pig immunized with chick cochlear tissue was uniformly elevated (Fig. 3).

The mean absorbance activity at a serum dilution of 1:250 was greater than 3 times the activity observed in control subjects; pooled preimmunization serum (1.573 ± 0.013 compared to 0.512 ± 0.025). Significant antibody cross-reactivity occurred when the sera obtained from guinea pigs immunized with chick cochlea were incubated against ELISA wells coated with guinea pig cochlea tissue (0.763 ± 0.153 at serum dilution of 1:250), (Fig. 4).

Similar cross-reactivity was not observed when wells coated with guinea pig cochlear tissue were incubated with pooled pre-immunization serum (0.226 ± 0.053 at serum dilution of 1:250).

In the mouse group, higher antibody activity was seen on the mice immunized with chick cochlea than in the mice immunized with guinea pig cochlea.

**Immunocytochemistry**

Labeled antibody bound to the stereocilia of organ of Corti hair cells was seen in cryosection incubated with sera obtained from guinea pigs immunized with chick cochlear antigen, using immunoperoxidase technique. Absence of stereocilia antibody binding was seen in the control group that was injected with preimmunization sera (Fig. 5,6). Nonspecific background staining occurred in the extralabyrinthine tissues and vestibular neuroepithelium in both experiment and control group.

Such immunoreaction did not show specific labeling with using an immunofluorescence technique. This may be because preservation of hair cell morphology was difficult to maintain with this technique, even when decalcification and cryoprotection steps were employed.

**Histology**

Prior to undergoing processing for pathological embedding, all specimens were examined to exclude middle ear infection or effusion. In a single guinea pig immunized with chick cochlea, thickened middle ear mucosa with post-inflammatoriy adhesions in the hypotympanum was found in one ear.

Six out of eight guinea pigs immunized with chick cochlea had mild hydropic change of scala media at mid modiolar area. These six animals demonstrated significant hearing loss of 25 to 35 dB.

In the mice immunized with guinea pig cochlea, hair cell degeneration in the middle and
apical twins of the cochlea revealed in only one animal.

Discussion
The results of our study included hearing loss coincident with immunization, cross-immune reaction, immunobinding of serum of animals to cochlear antigens, and evidence of cochlear pathology.

Significant hearing loss, defined as an elevation in hearing threshold of two standard deviations above of the mean threshold value for the control group, occurred in seven of eight guinea pigs immunized with chick cochlear. Varying degrees of amplitude and hearing threshold from ABR were evident. Comparing the hearing results in the mice immunized with chick cochlea, the hearing loss developed greater than did mice immunized with guinea pig cochlea. It may be concluded that cross-species immunization produced a more robust immune response in these animals.

However, this intraspecies variability is not unexpected given that each animal may possess different permeability characteristics of the blood-labyrinthine barrier.

To determine the antibodies to endogenous cochlear tissue by ELISA, significant antibody cross-reactivity occurred when the sera obtained from guinea pigs immunized with chick cochlea were incubated against ELISA wells coated with guinea pig cochlea. With the evidence from immunoperoxidase, the finding of antibodies bound to stereocilia of the organ of Corti and saccule, we may conclude that the cross-antibody to endogenous cochlea did exist. The binding of these antibodies may produce alterations in stereocilia activity, contractility characteristics, or transmembrane ionic gradients, which may lead to sensorineural dysfunction and resultant hearing loss.(12,15,16)

Of interest was the absence of modiolus vessel vasculitis, lymphocytic infiltration and inflammatory precipitate in the scala media. Such lack of pathology in the majority of the cochlea of immunized animals suggests that the interaction is subtle, non-lethal, and may perhaps be reversible.(17) The antisera showed immunostaining of guinea pig organ of Corti and saccule stereocilia, but not the utricle and ampulla stereocilia. The common embryologic origin of the cochlea and saccule might explain this finding.

The difficulty in interpretation by immunofluorescence methods were encountered similar to Soliman(18) due to the poor tissue preservation and processings. We suggests immunoperoxidase technique may be a better one.

Histological examination of temporal bone specimens did not reveal potential causes of conductive hearing loss. The inner ear findings of endolymphatic hydrops and organ of Corti degeneration in six animals are most consistent
with immune-mediated sensorineural hearing loss. (12,19,20) This may be the reversible histopathology causing clinical features mimic to Meniere’s pathology, and is supported by our finding in the guinea pigs immunized with chick cochlea which sustained significant hearing loss at 2,6 kHz seven days following each immunization have hearing return to normal at the third week prior to the third immunization.

![Graph](image)

**Fig. 1.** Hearing changes in the control guinea pig subject.

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**Fig. 2.** Hearing changes in the immunized guinea pig subject.

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**Fig. 3.** ELISA curve for serum from guinea pig immunized with chick cochlea vs. chick cochlear tissue plate.

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**Fig. 4.** ELISA curve for serum from guinea pig immunized with chick cochlea vs. guinea pig cochlear tissue plate.
Fig 5. Cryosection of guinea pig organ of Corti incubated with preimmunization serum from guinea pig (controls).

Fig 6. Cryosection of guinea pig organ of Corti incubated with serum from guinea pig immunized with chick cochlear tissue (horseradish peroxidase, original magnification x 100).

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IMMUNOLOGIC SENSORINEURAL HEARING LOSS
: การศึกษาในสัตว์ทดลอง

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บทคัดย่อ ผู้ว่ารายงานได้ทำการศึกษาเพื่อหาลักษณะภูมิคุ้มกันบัดกรรằngของยีนที่เกี่ยวข้องกับการเสื่อมการได้ยินชนิด sensorineural hearing loss (SNHL) โดยการศึกษารูปแบบภูมิคุ้มกันซึ่งสารสะท้อนจากผู้ได้รับการรักษาด้วยยาในการใช้ในหน่วยงานและหูหน้า ผลการศึกษาพบว่าผู้ที่มีการใช้ยา 7 ใน 8 สามารถตรวจพบการมีการรักษาด้วยยาในหน่วยงานและหูหน้า และยังพบการมีการคัดกรองตัวอย่างที่มีการใช้ยาในหน่วยงาน และมีการนับจำนวนเซลล์ที่เสียหายไปที่ผู้ที่ได้รับการรักษาด้วยยา ในหน่วยงานและหูหน้า โดยวิธีการที่ใช้เป็นการใช้ Immunohistochemistry และการสังเกตการณ์การ ใช้ immuno-staining ที่ hair cell stereocilia ได้ ภาพถ่ายแบบทางประสิทธิภาพใน organ of Corti ของเซลล์เกี่ยวกับการ Endolymphatic hydrops ซึ่งเกี่ยวกับการศึกษาแบบ cross-species immunization และผู้ที่ไม่ได้รับการให้ยา ทำให้ผู้เดิมที่ได้รับการรักษาด้วยยา มีการศึกษาการได้ยินชนิด SNHL ได้ เชียงใหม่ เวชสาร 2535;31: 183-192.