Scanning Microscopy

Volume 7 | Number 3 Article 15

6-29-1993

Immunological Pathogenesis of Endolymphatic Hydrops and Its Relation to Meniere's Disease

S. Tomiyama
Nippon Medical School

T. Yagi
Osaka University

M. Sakagami
Osaka University

K. Fukazawa
Osaka University

Scanning Microscopy, Vol. 7, No. 3, 1993 (Pages 907-920) 0891-7035/93$5.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

IMMUNOLOGICAL PATHOGENESIS OF ENDOLYMPHATIC HYDROPS AND ITS RELATION TO MENIERE'S DISEASE

S. Tomiyama[1,*], T. Yagi[2], M. Sakagami[2], and K. Fukazawa[3]

[1]Department of Otolaryngology, Nippon Medical School, Tokyo, Japan
[2]Department of Otolaryngology, School of Medicine, Osaka University, Osaka, Japan

(Received for publication March 5, 1993, and in revised form June 29, 1993)

Abstract

This study was designed to investigate an immunologically induced endolymphatic hydrops (ELH) and to focus on the issue of its pathogenesis in relation to Meniere's disease. The time course of ELH was evaluated by light microscopy in a 2-hour to 7-month period following direct antigen challenge to the endolymphatic sac (ELS) in systemically pre-sensitized guinea pigs. ELH began to appear in the vestibule and the basal turn 5-7 hours after inner ear challenge and developed gradually. During the interval from the second day to the first week, ELH rapidly developed in all the cochlear turns and reached a maximum size. During the period from the second week to the eighth week, ELH gradually reduced. After 9 weeks, ELH of the saccule and the cochlea gradually recurred. During the interval from the first week to the eighth week, the time course of ELH correlated well with the grade of cellular infiltration of the perisaccular tissue. These results suggest that recurrent immunological reaction in the ELS may result in disorders of the ELS which finally lead to the onset of Meniere's disease.

Key Words: Endolymphatic hydrops, Meniere's disease, endolymphatic hydrops, immune response, light microscopy, guinea pig.

*Address for correspondence:
S. Tomiyama
Department of Otolaryngology,
Nippon Medical School,
1-1-5 Sendagi, Bunkyo,
Tokyo 113, Japan
Telephone number: (03) 3822 2131
FAX number: (03) 5685 0830

Introduction

Endolymphatic hydrops (ELH) which constitutes an increase in the volume of endolymph, has been considered to result from an imbalance in secretion as well as from resorption of endolymph. It has been reported that ELH occurs in association with various diseases (Lindsay *et al.*, 1967; Schuknecht and Gulya, 1983), such as otitis media, trauma, collagen disease, syphilitic labyrinthitis, in which the inner ear disorder is characterized by individual pathogenesis. Accordingly, ELH has no single specific etiology. On the other hand, idiopathic ELH has been a significant pathological finding in Meniere's disease. Pathological studies of temporal bones of patients with Meniere's disease (Hallpike and Cairns, 1938; Arenberg *et al.*, 1970; Schuknecht, 1976) have ascertained that fibrotic degeneration is consistently observed in the endolymphatic sac (ELS), suggesting that ELH is principally caused by disorders of the ELS. In order to elucidate the pathogenesis of ELH accompanied by symptomatology of Meniere's disease, an animal experimental model is essential. In 1965, 100% induction of consistent and progressive ELH was first successfully demonstrated in the guinea pig by obliteration of the ELS (Kimura and Schuknecht, 1965). In this model, low tone hearing loss occurs in company with the time course (Suh and Cody, 1974; Konishi and Kelsey, 1976), while attacks of vertigo rarely appear. However, Kimura's model has been preferred for investigating the nature of ELH, since no superior animal model has been developed. Recently, fluctuating threshold elevation associated with the onset of cochlear ELH in Kimura's model was specifically demonstrated using a chronic implanted electrode (Aran *et al.*, 1984; Horner and Cazals, 1986), and also impairments of vestibular function were detected by rotational stimulation reported by Aran *et al.* (1984) and Horner *et al.* (1989). From the observation of Kimura's model, the sudden ablation of the ELS may represent a remission stage or end stage of Meniere's disease, but is unlikely to represent an active stage featuring attacks of vertigo. Therefore, a new ELH model incorporating attacks of vertigo and fluctuating hearing loss has been in considerable demand.

Fibrotic degeneration of the ELS seen in patients

with Meniere's disease may possibly suggest that chronic inflammatory reaction and/or recurrent immune reaction might have taken place in the ELS. Clinical evidence related to immunological etiology in Meniere's disease was first reported by Duke (1923). Recently, a basic study of the inner ear immunology has elucidated that the ELS is the only site in the inner ear in which immuno-competent cells capable of mounting immune reaction to pathogens and foreign bodies can be found (Rask-Andersen and Stahle, 1979, 1980; Arnold *et al.*, 1984; Wackym *et al.*, 1987; Takahashi and Harris, 1988; Altermatt *et al.*, 1990). Experimental studies have elucidated that the inner ear is independently capable of mounting an immune response (Harris, 1983) in which the ELS plays the central role (Tomiyama and Harris, 1986). These facts indicate that immune response mounted in the ELS should produce contradictory effects on the inner ear: a positive effect brought on by an inner ear immuno-defense and a negative effect triggered by an inner ear immuno-injury.

Recent studies from our laboratory have demonstrated that an immune reaction mounted in the ELS of the guinea pig consistently induces ELH (Tomiyama *et al.*, 1991a; Tomiyama, 1992a) accompanied by episodes of spontaneous nystagmus (Tomiyama *et al.*, 1991b) and fluctuating hearing loss (Ikezono and Tomiyama, 1992; Gotoh and Tomiyama, 1992). Control studies, such as primary antigen challenge to the ELS without systemic pre-sensitization, intradural secondary immune response and phosphate buffered saline injection to the ELS, were incapable of inducing ELH. These results have indicated that physio-morphological changes in the inner ear are actually caused by secondary immune response of the ELS, suggesting that the ELS plays an important role in the pathogenesis of Meniere's disease.

In this article, we further investigate the time kinetics of the development of ELH from the very early stage of two hours to 30 weeks post-secondary antigen challenge to the ELS, and assess the relationship between the inflammatory reaction of the ELS and ELH formation. Immunological pathogenesis of ELH and its relation to Meniere's disease are also discussed.

Materials and Methods

Materials and methods for induction of ELH were previously described in detail (Tomiyama and Harris, 1989). Briefly, Hartley guinea pigs were systemically immunized with 500 μg Keyhole limpet hemocyanin (KLH)/complete Freund's adjuvant and boosted twice with KLH/incomplete Freund's adjuvant once in two weeks until a high serum anti-KLH was obtained. Two weeks later, under general anesthesia, KLH (100 μg/5 μl) was directly injected into the ELS of the animals via the intradural approach.

Histological examination for light microscopy was performed by acid formalin fixation through cardiac perfusion, decalcification in ethylenediaminetetraacetic acid (EDTA) with 2.5% paraformaldehyde, paraffin-embedding, serially sectioning at 4 μm thickness, and staining with hematoxylin and eosin.

For observation of the fine structure of the ELS by conventional thin sections, fixation was done *in-vivo* by vascular perfusion with a modified Karnovsky fixative: 1% paraformaldehyde and 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3). A further step for electron microscopy was carried out *ad modum* Graham and Karnovsky (1966). Temporal bone specimens of 277 animals were obtained from 2 hours to 7 months post-inner ear antigen challenge.

The extent of ELH was graded as follows.

The cochlea: mild size denotes slight convexity of Reissner's membrane; moderate size, intermediate between mild size and extensive size; and extensive size, marked convexity of Reissner's membrane which touches the bony partition between the turns.

The saccule: mild size denotes convexity of the membrane of less than half the distance between the normal position and the footplate; moderate size, convexity of the membrane of more than half the distance between the normal position and the footplate; and extensive size, membrane touching or very close to the footplate.

The utricle: the extent of utricular ELH was not graded.

Inflammatory reaction of the ELS is represented by the grade of cellular infiltration in the ELS. Cellular infiltration in the sac was graded as follows: Grade I denoting 1 to 10 cells per x400 high power field (hpf); II: 11 to 100 cells per hpf; III: more than 100 cells per hpf; IV: too numerous to count.

Statistical analysis between the grade of cellular infiltration in the ELS and the extent of ELH was evaluated by Spearman's correlation test.

Results

Development of ELH

The saccule: The time course of the incidence and extent of saccular ELH following secondary immune response in the ELS is shown in Table 1. In the period from 2 to 4 hours post-challenge, ELH was not observed. ELH gradually occurred after 5 to 7 hours and developed into a small size in 9 out of 10 ears in the 12 hour specimens. Thereafter, ELH rapidly reached a maximum size within the first week. In the period from the 2nd week to the 7-8th week, the incidence of hydrops was gradually reduced to 29% at the 7-8th week. After the 9th week, hydrops gradually recurred which reached a 73% incidence at the 20-30th week with a predominant increase in mild size.

The cochlea: The time course of the incidence of cochlear ELH following secondary immune response in the ELS is shown in Figure 1. ELH formation of small size gradually started from the basal turn 2-4 hours post-inner ear challenge. ELH rapidly developed in all the cochlear turns and reached a maximum size on day 2.

Table 1. Time course of saccular endolymphatic hydrops following secondary immune reaction in the endolymphatic sac. Note: h = hour; d = day; w = week; n = number of animals; a = incidence of endolymphatic hydrops (%); b = extent of endolymphatic hydrops.

Survival time	2-4h	5-7	12	1d	2	4	1w	2	3	4	5-6	7-8	9-16	20-30
n	7	8	10	12	28	25	34	26	21	20	23	17	24	22
ELH[a]	0	25	90	75	90	92	97	69	67	65	52	29	67	63
mild[b]	0	25	80	67	54	56	41	50	62	30	26	17	33	45
moderate	0	0	10	8	36	20	41	11	5	15	13	12	21	9
extensive	0	0	0	0	0	16	15	8	0	20	13	0	13	9

Table 2. Time course of utricular endolymphatic hydrops following secondary immune reaction of the endolymphatic sac. Note: h = hour; d = day; w = week; n = number of animals; a = incidence of endolymphatic hydrops (%).

Survival time	2-4h	5-7	12	1d	2	4	1w	2	3	4	5-6	7-8	9-16	20-30
n	7	8	10	12	28	25	34	26	21	20	23	17	24	22
ELH[a]	0	12	30	42	50	48	50	38	14	30	35	12	17	36

After the first week, this acute developmental ELH gradually reduced in all the cochlear turns until the 5th week. In the period from the 7th week to the 30th week, ELH gradually recurred, preferentially in the basal and apical turns. Mild ELH increased in the basal turn, while moderate to extensive ELH comparatively increased in the apical turns. The time course of the incidence and extent of ELH in the individual cochlear turns were as follows:

Basal turn (Fig. 1a): Mild ELH was occasionally seen from 2 to 4 hours and obviously developed from 12 hours post-inner ear challenge. On day 2, ELH rapidly developed and reached a maximum size which persisted to the first week. In the period from the second week to the 6th week, it gradually reduced in size and incidence. From the 7th week, ELH gradually recurred. The incidence of mild ELH actually increased from 24% at the 7-8th week to 60% at the 20-30th week.

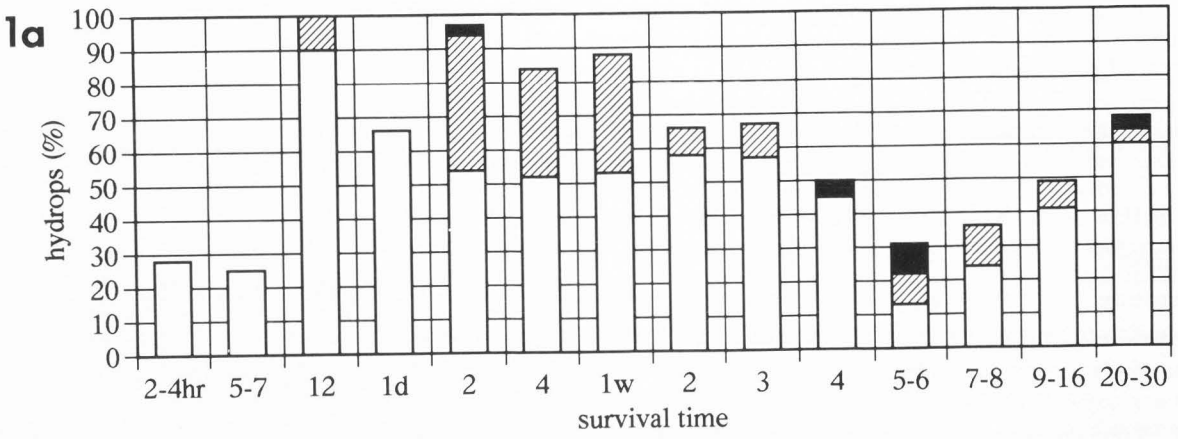
Second turn (Fig. 1b): In the interval from 2 to 7 hours post-inner ear challenge, ELH was not observed. In the 12 hour specimen, six out of ten ears showed minimal ELH. Thereafter it rapidly developed on day 2 in which the total incidence reached 85% with a 30% increase in the moderate to extensive size. The incidence and extent of ELH from day 4 to the first week maintained almost the same level as those of day 2. After the second week, it reduced in incidence and extent. In the period from the 4th week to the 30th week, the incidence of ELH did not increase and was maintained at around 25%, of which the moderate to extensive size persisted as compared to the mild size.

Third turn (Fig. 1c): Mild ELH began to form in six out of 10 ears after 12 hours. ELH rapidly developed from the second day to the fourth day. After the second week, it gradually reduced in incidence and extent until the 6th week, while extensive ELH persisted in a small number of animals. In the period from the 7th week to the 30th week, the incidence of ELH slightly increased.

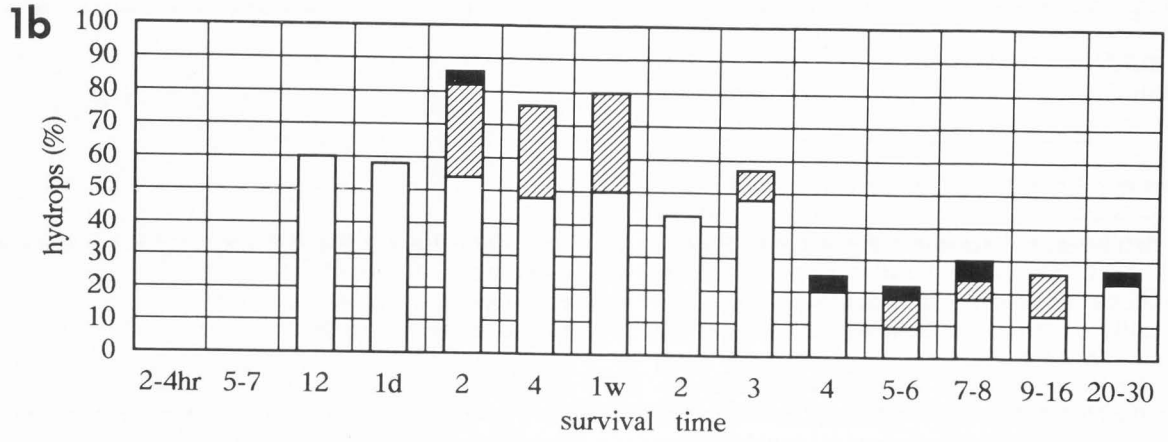
Fourth turn (Fig. 1d): Mild ELH initially developed in 3 out of 10 ears (30%) after 12 hours post-inner ear challenge. ELH rapidly developed on day 2 and reached a maximum size in the first week. In the period from the 2nd week to the 6th week, it gradually reduced in incidence. After the 9th week, ELH gradually recurred.

Apex (Fig. 1e) ELH was not seen in the period from 2 hours to 7 hours post-inner ear challenge. Mild ELH occasionally developed in the period from 12 hours to the first day. Initial ELH principally occurred from the 2nd day, developing until the first week. In the period from the 2nd week to the 6th week, it was gradually reduced in incidence from 50% to 24%, while moderate to extensive ELH persisted as compared to the mild size after the 4th week. In the period from the 7th week to the 30th week, it gradually recurred and was found in 64% of the animals at the 20-30th week increasing evenly from mild to extensive size.

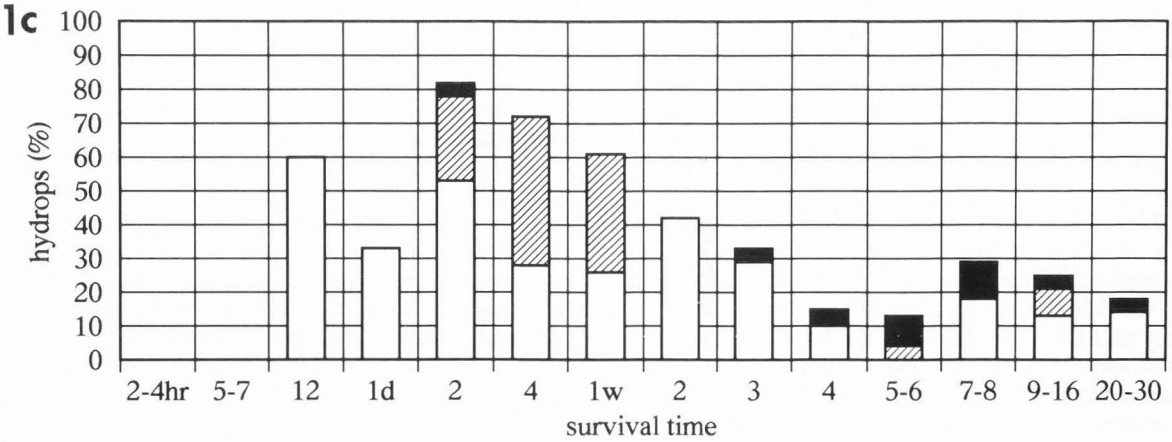
The utricle: The time course of utricular ELH is shown in Table 2. ELH gradually occurred after 5-7 hours and reached a maximum (50%) on day 2 which



(n) (7) (8) (10) (12) (28) (25) (34) (26) (21) (20) (23) (17) (24) (22)



(n) (7) (8) (10) (12) (28) (25) (34) (26) (21) (20) (23) (17) (24) (22)



(n) (7) (8) (10) (12) (28) (25) (34) (26) (21) (20) (23) (17) (24) (22)

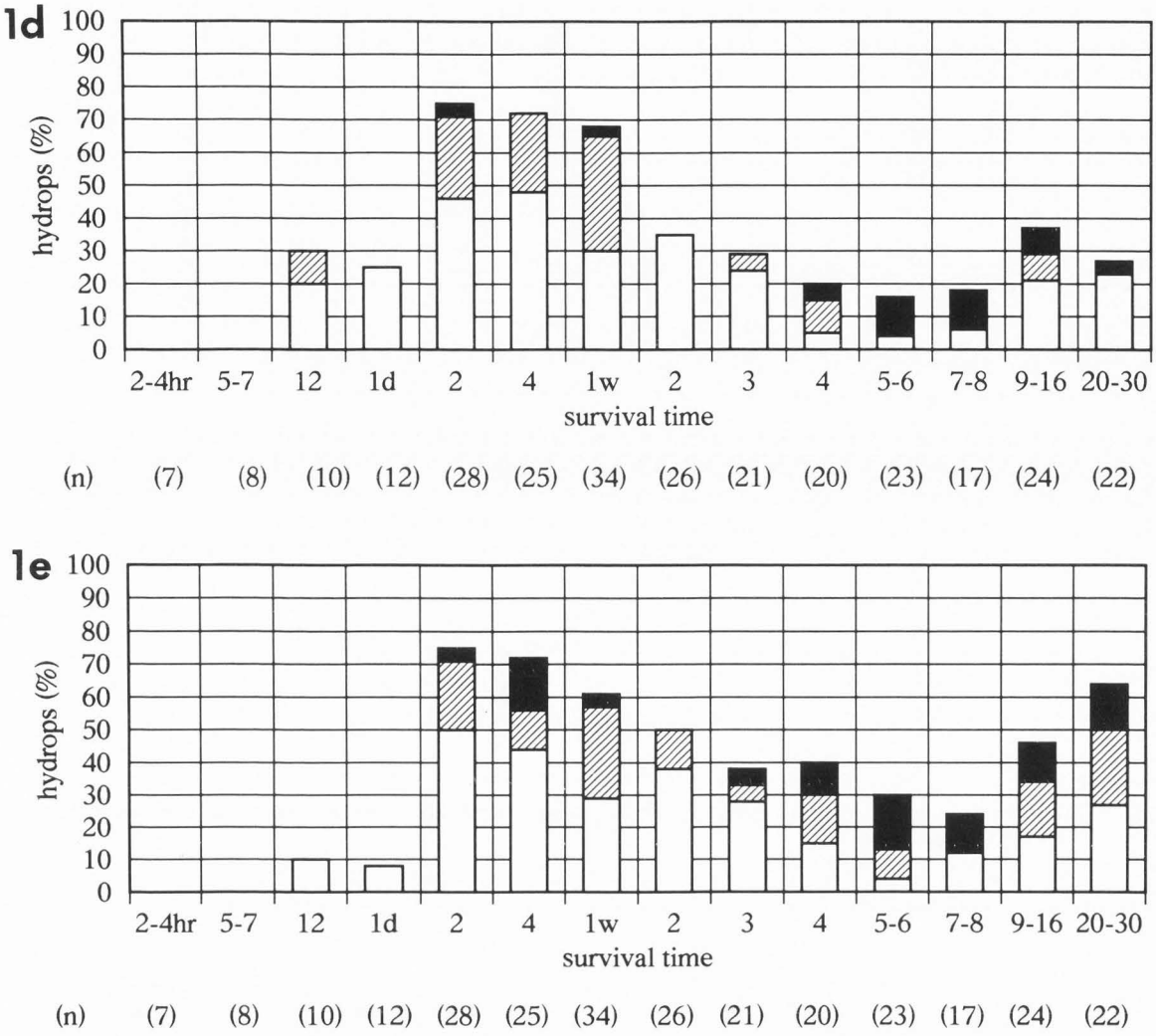


Figure 1. The time course of endolymphatic hydrops of individual cochlear turn. **1a**: the basal turn; **1b**: the second turn; **1c**: the third turn; **1d**: the fourth turn; and **1e**: the apical turn. Vertical axis: incidence of endolymphatic hydrops (%). hr: hour; d: day; w: week; n: number of animals. Extent of endolymphatic hydrops: □: mild; ▨: moderate; and ■: extensive.

persisted until the first week. After the first week, ELH was slightly reduced. The incidence of utricular ELH demonstrated no definite increase along with the time course.

Cellular infiltration of the ELS

The time course of the grade of cellular infiltration within the ELS is shown in Table 3. In the period from 2 to 4 hours, a small number of inflammatory cells, mainly polymorphonuclear cells (PMNs) and macrophages, infiltrated in the lumen of the ELS and the perisaccular tissue. Perivascular cuffing was often observed in the distal portion close to the lateral sinus. The grade of cellular infiltration increased and reached grade IV at 12 hours post-inner ear challenge and persisted to the second day. After the fourth day, the grade of cellular infiltration gradually decreased. In the period from the 4th day to the first week, PMNs and macrophages dramatically decreased in number, while lymphocytes and plasma cells increased. After the first week, infiltrative cells were predominant in plasma cells and lymphocytes which were preferably seen in the perisaccular tissue. Even in the period from the 9th week to the 30th week, infiltration of plasma cells and lymphocytes, ranked as grade II, was observed in 54-64% of the animals. Several of the other animals demonstrated moderate cellular infiltration of grade III. However, neither fibrotic degeneration nor atrophy of the epithelial cells of the ELS were identified under light microscopic observation.

Table 3. Time course of cellular infiltration in the endolymphatic sac following secondary immune reaction of the endolymphatic sac. Note: h = hour; d = day; w = week; n = number of animals; a = grade of cellular infiltration in the endolymphatic sac (%).

Survival time	2-4h	5-7	12	1d	2	4	1w	2	3	4	5-6	7-8	9-16	20-30
n	7	8	10	12	28	25	34	26	21	20	23	17	24	22
I[a]	0	0	0	0	0	0	0	4	10	10	31	6	29	27
II	71	0	0	0	3	12	12	35	43	55	52	65	54	64
III	29	100	0	0	7	20	44	46	43	35	17	29	17	9
IV	0	0	100	100	90	68	44	15	4	0	0	0	0	0

Table 4. Correlation coefficient between the extent of endolymphatic hydrops and the grade of cellular infiltration in the endolymphatic sac. Note: a = the extent of saccular endolymphatic hydrops (ELH) versus (vs) the grade of cellular infiltration of the endolymphatic sac (ELS); b = the extent of ELH of the basal turn vs the grade of cellular infiltration of the ELS;, c = the extent of ELH of the apical turn vs the grade of cellular infiltration of the ELS; w = week; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$ (Spearman's correlation); n.s. = not statistically significant.

Survival time	number of animals	Saccule[a]	Basal turn[b]	Apical turn[c]
1-4w	101	0.3585***	0.3912***	0.3550***
5-6w	23	0.572 **	0.4473 *	0.6869***
7-8w	17	0.4910 *	0.5877 *	0.4910 *
9-16w	24	0.4420 *	0.2117 n.s.	0.2903 n.s.
20-30w	22	0.0459 n.s.	-0.2129 n.s.	0.1422 n.s.

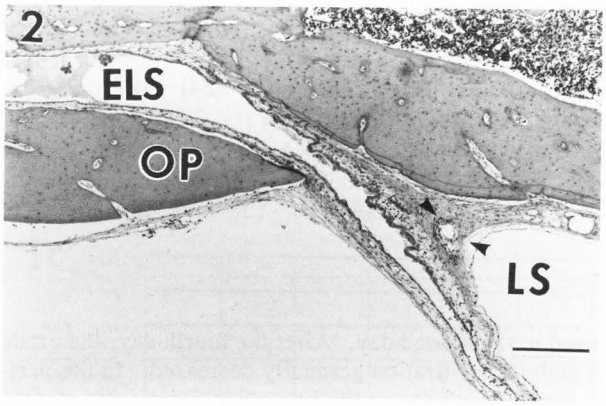
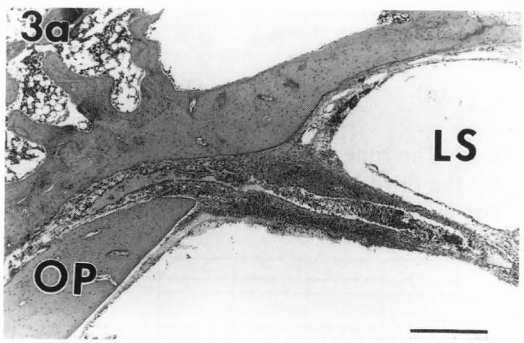


Figure 2. Two hour survival time. Mild cellular infiltration is seen in the perisaccular space. Arrow = perivascular cuffing; ELS = endolymphatic sac; OP = the operculum; LS = the lateral sinus. Bar = 100 μm.



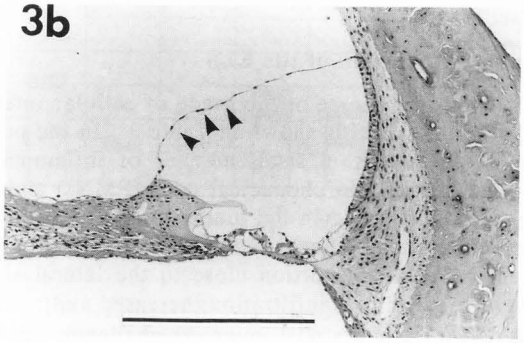
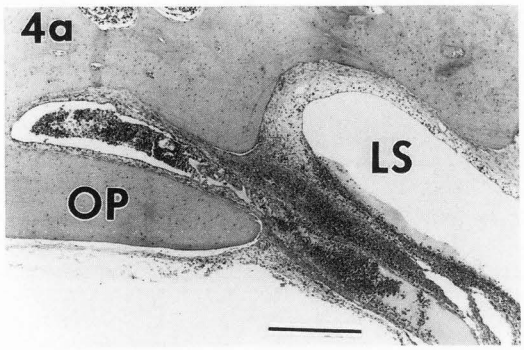
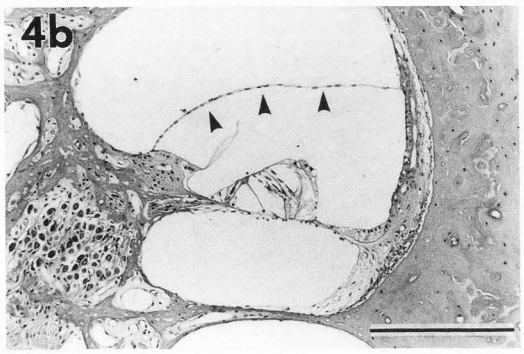
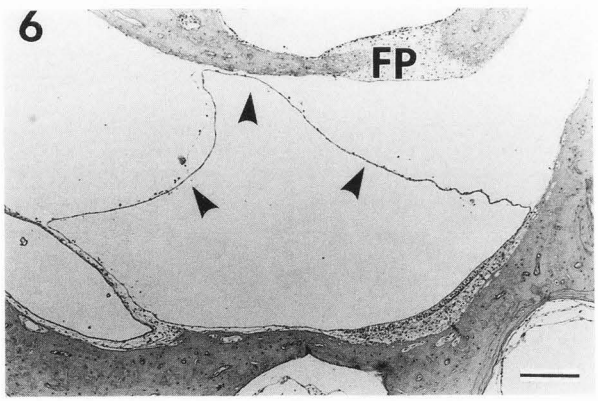
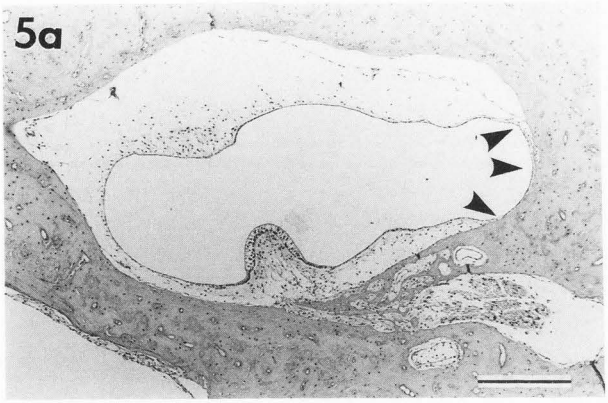


Figure 3. Five hour survival time. **a**: A great number of inflammatory cells seen in the lumen of the endolymphatic sac and the perisaccular tissue. OP = the operculum; LS = the lateral sinus. **b**: The same specimen shown in Figure 3a. Mild hydrops is seen in the basal turn (arrows). Bars = 100 μm.









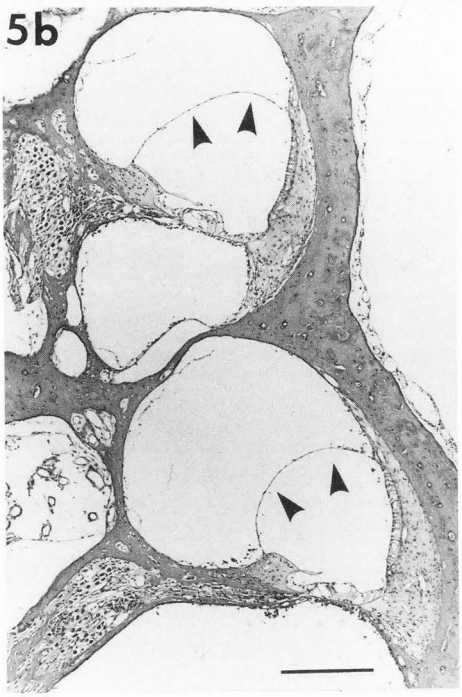
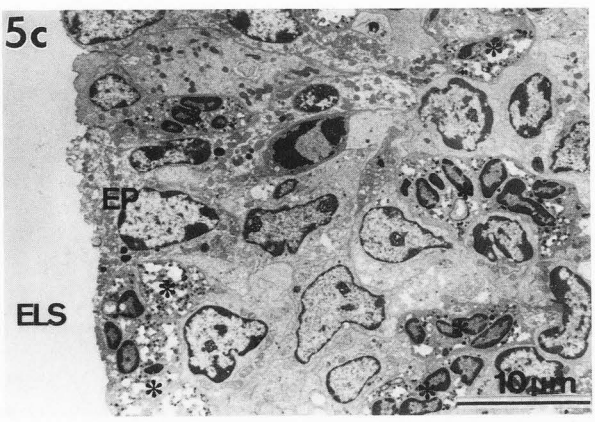
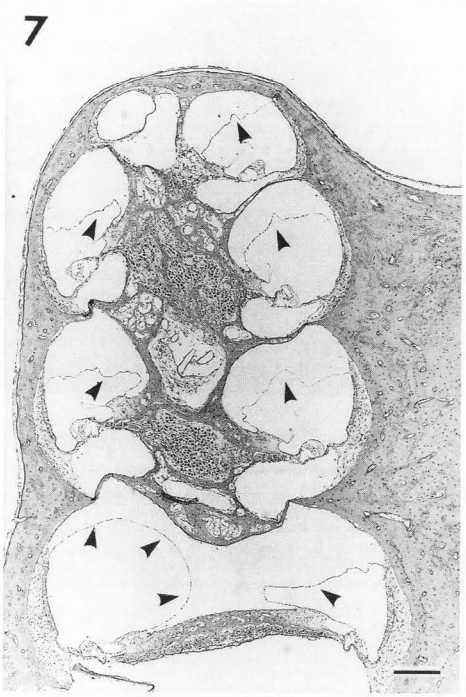


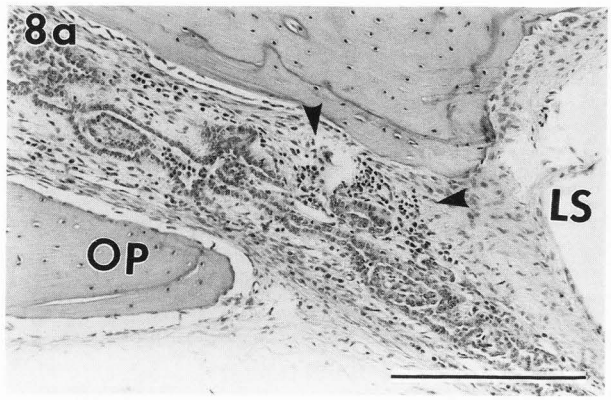
Figure 4. Twelve hour survival time. **a**: Numerous inflammatory cells in the endolymphatic sac. OP = the operculum; LS = the lateral sinus. **b**: The same specimen shown in Figure 4a. Mild hydrops is seen in the fourth turn (arrows). Bars = 100 μm.

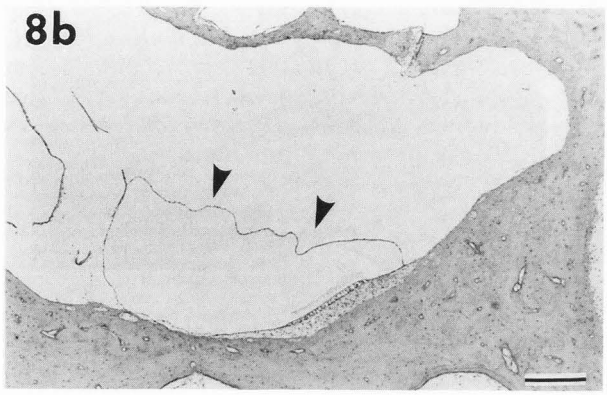
Figure 5. Two day survival time. **a**: Utricular membrane is in close proximity to the bony wall (arrows). Bar = 100 μm. **b**: The same specimen shown in Figure 5a. Moderate hydrops in the basal turn and mild hydrops in the second turn are seen (arrows). Bar = 100 μm. **c**: Electron micrograph of the endolymphatic sac. Many polymorphonuclear cells(*) are found between epithelial cells (EP) and in the subepithelial space. ELS = the lumen of the endolymphatic sac. Bar = 10 μm.

Figure 6. Four day survival time. Extensive hydrops of the saccule is seen (arrows). FP = Footplate of the stapes. Bar = 100 μm.









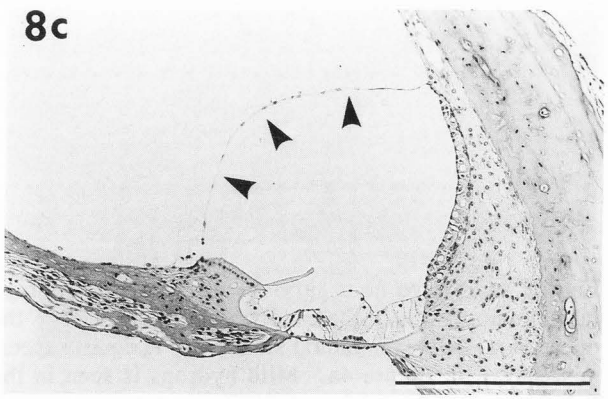


Figure 7. One week survival time. Moderate hydrops with collapse (arrows) is seen in the cochlea. Bar = 100 μm.

Figure 8. Four week survival time. **a**: A moderate number of plasma cells and lymphocytes is seen in the perisaccular tissue (arrows). OP = the operculum; LS = the lateral sinus. **b**: The same specimen shown in Figure 8a. Mild hydrops accompanied by collapse (arrows) is seen in the saccule. **c**: The same specimen shown in Figure 8a. Moderate hydrops is seen in the basal turn. Bars = 100 μm.

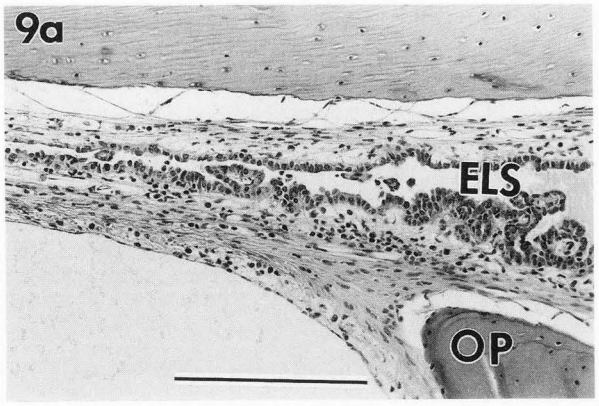
Relation between the grade of cellular infiltration in the ELS and the extent of ELH in the pars inferior

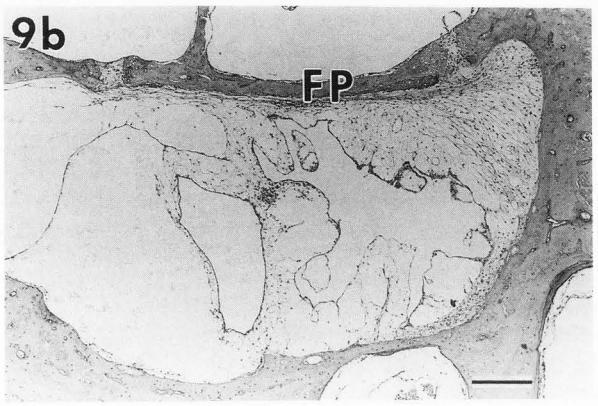
In order to elucidate whether or not prolonged reaction in the ELS impaired endolymph absorption of the ELS, the correlation between the grade of cellular infiltration in the ELS and the degree of ELH was examined (Table 4). In the first eighth week period, a significant correlation was seen in ELH of the saccule, basal and apical turns. In the 9th to 16th week period, a significant correlation was seen in ELH of the saccule, but not in that of the basal and apical turns. In the 20th to 30th week period, no significant correlation was seen in ELH of the saccule, basal and apical turns.

The time course of morphological changes of the inner ear is shown in Figures 2 to 10.

Discussion

The immunological approach to pathogenesis of Meniere's disease has been developed by clinical evidence based on immunological etiology. Duke (1923) first reported several cases who suffered attacks of vertigo by eating certain foods, suggesting an induction of Meniere's disease by exogenous antigenic materials. Lehnhardt (1958) first reported cases of bilateral sudden deafness and proposed the etiological hypothesis that the degeneration in the organ of Corti resulted in producing an anti-cochlea antibody with which another organ of Corti reacted as the antigen in the individual. Kikuchi (1959) also reported several cases in which hearing in





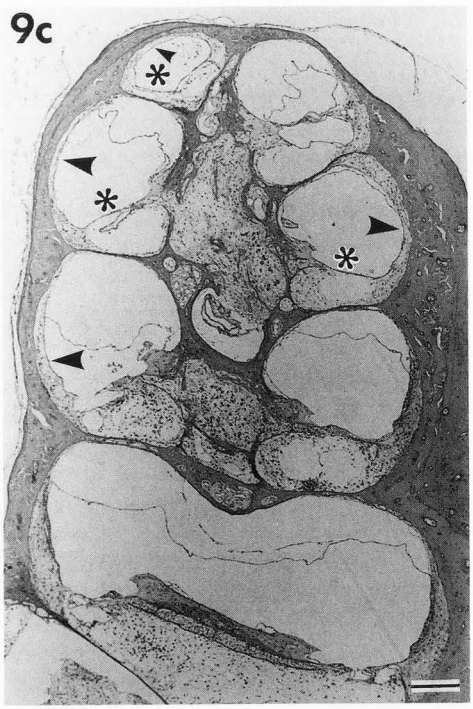


Figure 9. Twenty week survival time. **a**: A small number of lymphocytes and plasma cells is seen in the perisaccular tissue. OP = the operculum; LS = the lateral sinus. Bar = 50 μm. **b**: The same specimen shown in Figure 9a. The fibrotic change is seen in the perilymphatic space of the vestibule. FP = footplate of the stapes. Bar = 100 μm. **c**: The same specimen shown in Figure 9a. Moderate to extensive hydrops accompanied by severe degeneration of the cochlear sensory cells is seen in the cochlea. The total loss of the organ of Corti (*), loss of spiral ganglions (arrow), atrophy of the stria vascularis (arrow) and fibrotic proliferation of the scala tympani of the second turn are found. Bar = 100 μm.

---

the opposite ear had been markedly influenced by operation on the other ear. These reports suggest an induction of Meniere's disease by endogenous antigen, namely autoimmune disease. Consequently, the immunological approach to Meniere's disease should be principally classified into two types by antigen character: one is by exogenous antigen and the other is by endogenous antigen.

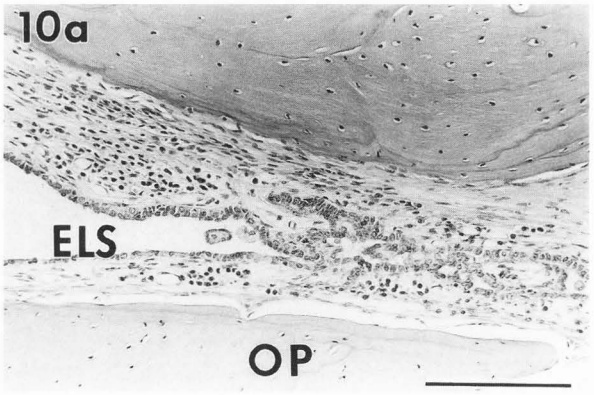
The purpose of the present study was then to elucidate whether or not an inner ear immune response in reaction to exogenous antigens is capable of inducing ELH similar to Meniere's disease.

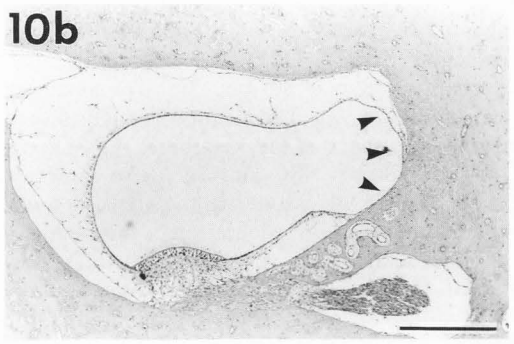
Type I allergic reaction related to Meniere's disease has been suspected in previous clinical reports (Duke, 1923; Dohlman, 1939; Shambaugh and Roberts, 1942; House and Powers 1969). Currently, however, a definite correlation between type I allergic reaction and Meniere's disease has not been confirmed, since patients with Meniere's disease demonstrate normal serum levels of IgE (Stahle, 1976), and also patients with attacks of vertigo due to certain foods maintained normal serum levels of antigen specific IgE levels (Hozawa, 1980).

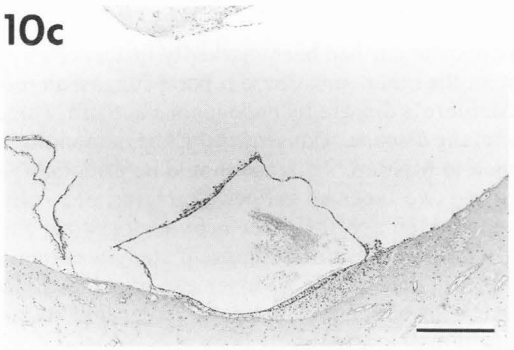
Consequently, a type III allergic reaction has been preferentially suggested to be the immunological etiology of Meniere's disease. Several methods of antigen challenge to the inner ear have been explored for eliciting an inner ear immune reaction, such as the middle ear cavity, stylomastoid foramen, intradural space, scala tympani, and ELS.

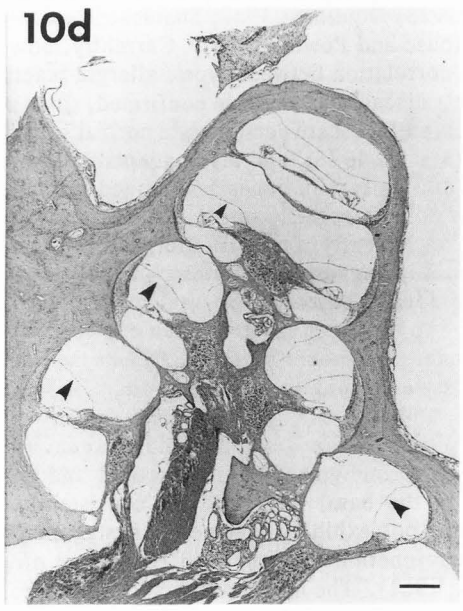
Antigen challenge to the middle ear cavity in presystemically immunized animals could induce ELH, mainly in the basal turn, but not consistently. This model did not exhibit spontaneous nystagmus, though vestibular function was impaired (Owada *et al.*, 1960; Hazama, 1961). The model principally generates immune reaction in the middle ear which causes an increased permeability of the inner ear membrane and the cochlear venule, and permits the immune products, antigen itself and plasma, to infiltrate into the inner ear (Saijo and Kimura, 1984; Harris *et al.*, 1986a). Otitis media accompanied with ELH has been clinically noted (Paparella *et al.*, 1979).

Recurrent antigen inoculation through the stylomastoid foramen in systemically pre-sensitized animals









induced ELH accompanied by spontaneous nystagmus (Kumagami *et al.*, 1976). Their study demonstrated similar symptoms of Meniere's disease. Further studies are necessary to elucidate the relationship between inner ear immune response and inner ear disorder.

Direct antigen challenge into the perilymphatic space of the cochlea in systemically pre-sensitized guinea pigs could induce ELH (Tomiyama and Harris, 1986). However, immune reaction in the perilymphatic space caused severe degeneration of the cochlear sensory tissue and the animals demonstrated irreversible hearing loss (Woolf and Harris, 1986). These results suggest that antigen presentation into the cochlea is not suitable in inducing ELH similar to Meniere's disease.

Intradural antigen challenge in systemically pre-sensitized animals did not induce ELH or inner ear impairment (Tomiyama *et al.*, 1993).

Tomiyama and colleagues have reported that direct antigen challenge to the ELS in systemically pre-sensitized guinea pigs consistently induced ELH accompanied with symptomatology of Meniere's disease (Tomiyama *et al.*, 1991a, 1991b; Tomiyama, 1992a; Nonaka *et al.*, 1992; Ikezono and Tomiyama, 1992; and Gotoh and Tomiyama, 1992). This model is based on the type III allergic reaction, since a histological study has demonstrated generation of IgG and C_3 complement (Takahashi and Tomiyama, 1990) and acute inflammatory reaction in the ELS (Tomiyama *et al.*, 1991a). The present study further confirms the existence of a close relationship between the time course of ELH and cellular infiltration in the ELS in the period from 2 hours to 30 weeks post-inner ear antigen challenge.

From 2 to 12 hours post-inner ear challenge, ELH of pars inferior gradually occurred after 5-7 hours. At 12 hours, ELH increased to an incidence of 90% in the saccule and 100% in the basal turn with a maximum increase (grade IV) of cellular infiltration in the ELS. This result indicated that development of ELH was coincident with the time course of inflammatory reaction in the ELS.

In the period from the second day to the first week, ELH rapidly developed to all of the cochlear turns and reached a maximum size. The mechanism of rapid development of ELH is assumed to be due to an excess

Figure 10. Twenty-eight week survival time. **a**: A small number of lymphocytes and plasma cells (ranked as grade II) is seen in the perisaccular tissue. ELS = the endolymphatic sac lumen; OP = the operculum; LS = the lateral sinus. Bar = 50 μm. **b**: Utricular hydrops is seen (arrows). Bar = 100 μm. **c**: moderate hydrops of the saccule is seen. Bar = 100 μm. **d**: Mild hydrops is seen from the second to fourth turns (arrow). Bar = 100 μm.

production of endolymph as well as to an impaired absorption. The present study demonstrated a vigorous accumulation of inflammatory cells in the ELS lumen and perisaccular tissue from the 12th hour to the 2nd day, suggesting a disorder of ELS function as one of the courses of ELH in this model. Previously, concomitant elevation of serum antibody marker in the perilymph was demonstrated on day 2, and the grade paralleled perilymph antigen specific antibody levels within the first week, suggesting an increased vascular permeability of the inner ear as one of the courses of ELH (Tomiyama, 1992b). Additionally, these findings have been supported by an electron microscopic study which demonstrated a significant leakage of horseradish peroxidase from the stria vascularis of the challenged ear as compared to the contralateral untreated ear (Sakagami *et al.*, 1991).

After the first week, ELH was gradually reduced until the 5th-8th week. In this period, factors that induced rapid hydrops formation, as mentioned above, disappeared so that ELH was gradually reduced in size. In this period, ELH was still found in the ear in which moderate to severe cellular infiltration persisted in the perisaccular tissue. This result indicates that the pace of reduction of ELH may depend on the degree of recovery of ELS function.

In the 9th to 30th week period, ELH gradually recurred in the saccule, basal turn, and apical turns. This result suggests that chronic disorder of the ELS may slightly impair resorption of endolymph. Fibrotic proliferation in the vestibular space was occasionally seen in this stage. However, there was no statistically significant correlation between the grade of cellular infiltration in the ELS and the extent of ELH in this period, except for the saccular ELH in the 9th to 16th week period. In light microscopic observations, neither loss of vascular network of the perisaccular tissue, nor atrophy of ELS epithelium, nor fibrotic degeneration were clearly identifiable. Further study is needed to verify whether or not irreversible degeneration occurs in the epithelium of the ELS and in the perisaccular tissue.

The time course of utricular ELH was not the same as that of the pars inferior. The incidence of ELH in the pars superior was less than that of the pars inferior. This result is the same as Kimura's reported ELH model (Kimura and Schuknecht, 1965).

The present study demonstrated that an immune reaction in the ELS can induce ELH. It then remains to be known how foreign bodies, including pathogens, actually enter the sac. The ELS freely communicates with the systemic circulation via the fenestrated vessels (Lundquist, 1965; Rask-Andersen *et al.*, 1983), indicating an active trapping of foreign bodies by the ELS.

This study employed Hartley guinea pigs which are not syngeneic. Therefore, immune reaction is individually different so that the time kinetics of ELH in this model largely depends on the strength on the immune response of the ELS. Immunological analysis for mechanisms of ELH formation will be clearer if syngeneic animals are employed.

Coming to the point then of autoimmune etiology, guinea pigs systemically sensitized with homologous crude inner ear antigen have demonstrated hearing disturbance in which the main histological changes were degeneration of the spiral ganglion and stria vascularis, whereas ELH was not significant (Beikert, 1961; Terayama and Sasaki, 1964). However, systemic immunization with stria vascularis obtained from normal rabbits, induced ELH in normal guinea pigs, but not in the complement 4 deficiency guinea pigs, suggesting a relationship between the immune complex and the development of ELH (Harada *et al.*, 1984).

Yoo *et al* (1982) have proposed that autoimmunity to type II collagen may be of etiological importance in otosclerosis and Meniere's disease. This hypothesis is based on highly elevated anti-type II collagen serum levels in patients with both diseases as compared to those of normal controls. Yoo *et al.* (1983a, b) have also experimentally demonstrated ELH with impaired inner ear function by systemic immunization using homologous type II collagen. The appearance of spontaneous nystagmus has not yet been observed in the autoimmune experimental studies while vestibular function is impaired. They hypothesized that ELH is induced by disorder of the ELS and duct through immune reaction with type II collagen in the inner ear, which leads to attacks of vertigo when the endolymphatic membrane ruptures. However, Harris *et al.* (1986b) have reported that a reexamination of type II collagen autoimmunity did not produce an autoimmune inner ear disorder. Currently, specific antigenicity for inner ear tissue including type II collagen is still under investigation. Further study is needed to elucidate the true inner ear antigen.

In summary, the present study demonstrated that immune reaction mounted in the ELS induced a rapid formation of ELH and recurrent ELH with gradual progression. It may be possible to hypothesize that recurrent antigen trapping in the ELS causes immune injury to it and to inner ear function, which induces ELH accompanied by an attack of vertigo similar to Meniere's disease.

References

Altermatt HJ, Gebbers JO, Muller C, Arnold W, Laissue JA (1990) Human endolymphatic sac: evidence for a role in inner ear immune defense. ORL **52**, 143-148.

Aran JM, Rarey KE, Hawkins Jr JE (1984) Functional and morphological changes in experimental ELH. Acta Otolaryngol (Stockh) **97**, 547-557.

Arenberg IK, Marovitz WF, Shambaugh Jr GE (1970) The role of the endolymphatic sac in the pathogenesis of endolymphatic hydrops in man. Acta Otolaryngol Suppl, **275**, 7-49.

Arnold W, Altermatt HJ, Gebbers JO, Laissue JA (1984) Secretory immunoglobulin A in the human endolymphatic sac. ORL **46**, 286-288.

Beikert P (1961) Zur frage der Empfindungsschwerhorigkeit und Autoallergie (Aspect of sensory

hearing loss and autoimmunity). Z Laryng Rhinol Otol **40**, 837-842.

Dohlman G (1939) Menieresche Krankheit auf allergisher Basis (Meniere's disease on allergic base). Acta Otolaryngol **27**, 245-248.

Duke WW (1923) Meniere's syndrome caused by allergy. JAMA **81**, 2179-2181.

Gotoh Y, Tomiyama S (1992) Immunoinjury to the inner ear auditory system following secondary endolymphatic sac immune response. J Otolaryng Jpn. **95**, 963-968.

Graham RC, Karnovsky MJ (1966) The early stages of absorption of injected horseradish peroxidase in the promaximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. J Histochem Cytochem **14**, 291-302.

Hallpike CS, Cairns H (1938) Observations on the pathology of Meniere's syndrome. J Laryngol Otol **53**, 625-654.

Harada T, Matsunaga T, Hong K, Inoue K (1984) Endolymphatic hydrops and III type allergic reaction. Acta Otolaryngol (Stockh) **97**, 450-459.

Harris JP (1983) Immunology of the inner ear: Response of the inner ear to antigen challenge. Otolaryngol Head Neck Surg **91**, 17-23.

Harris JP, Keithley EM, Ryan AF (1986a) Passage of antibody from the middle ear into the inner ear. In: Acute and Secretory Otitis Media. Kugler Publ., Amsterdam. 241-246.

Harris JP, Woolf NK, Ryan AF (1986b) A reexamination of experimental type II collagen autoimmunity: middle and inner ear morphology and function. Ann Otol Rhinol Laryngol **95**, 176-180.

Hazama H (1961) Experimental study on peripheral circulatory disturbances and inner ear changes due to local allergic reaction. J Otolaryng Jpn **64**, 690-703.

Horner KC, Cazals Y (1986) Rapidly fluctuating thresholds at the onset of experimentally-induced hydrops in the guinea pig. Hear Res **26**, 319-325.

Horner KC, Erre JP, Cazal Y (1989) Asymmetry of evoked rotatory nystagmus in the guinea pig after experimental induction of endolymphatic hydrops. Acta Otolaryngol Suppl **468**, 65-69.

House WF, Powers WH (1969) The dizzy patient-allergic aspects. Laryngoscope **79**, 1331-1338.

Hozawa J (1980) Immunological considerations on recurrent episodes of Meniere's disease. Pract Otol (Kyoto) **73**, 527-539.

Ikezono T, Tomiyama S (1992) Effects of inner ear immune responses on auditory function in guinea pig. J Otol Jpn **95**, 809-816.

Kikuchi M (1959) On the "sympathetic otitis". Jibi Rinshyo Kyoto **52**, 600-605.

Kimura RS, Schuknecht HF (1965) Membranous hydrops in the inner ear of the guinea pig after obliteration of the endolymphatic sac. Pract Oto-rhino-laryng **27**, 343-354.

Konishi T, Kelsey E (1976) Cochlear potentials and electrolytes in endolymph in experimentally induced endolymphatic hydrops of guinea pig. In: Electrocochleography. Ruben RJ *et al.* (eds.) University Park Press, Baltimore, Maryland. pp. 295-313.

Kumagami H, Nishida H, Dohi K (1976) Experimental labyrinthine lesions through stylomastoid foramen. ORL **38**, 334-343.

Lehnhardt E (1958) Plötzliche Hörströrungen, auf beiden Seiten gleichzeitig order nacheiander aufgetreten (Sudden deafness, bilateral simultaneously or subsequently). Z Laryng Rhinol Otol **37**, 1-16.

Lindsay JR, Kohut RI, Sciarra PA (1967) Meniere's disease: pathology and manifestations. Ann Otol Rhinol Laryngol **76**, 5-22.

Lundquist PG (1965) The endolymphatic duct and sac in the guinea pig. An electron microscopic and experimental investigation. Acta Otolaryngol Suppl **201**, 1-108.

Nonaka M, Tomiyama S, Gotoh Y, Ikezono T, Yagi T (1992) Vestibular disorder following immune response of the endolymphatic sac in the guinea pig. Ann Otol Rhinol Laryngol **101** Suppl **157**, 54-57.

Owada K, Shiizu 5, Yamazaki Y (1960) An interpretation of the appearance of nystagmus in Meniere's disease. Acta Otolaryngol **52**, 486-492.

Paparella MM, Goycoolea MV, Meyerhoff WL (1979) Endolymphatic hydrops and odtis media. Laryngoscope. **89**, 43-58.

Rask-Andersen H, Stahle J (1979) Lymphocyte-macrophage activity in the endolymphatic sac. An ultrastructural study of the rugose endolymphatic sac in the guinea pig. ORL **41**, 177-192.

Rask-Andersen H, Stahle J (1980) Lymphocyte-macrophage interaction in the endolymphatic sac. Acta Otolaryngol **89**, 283-294.

Rask-Andersen H, Bagger-Sjoback D, Lundquist PG (1983) The fenestrated blood vessels of the endolymphatic sac. A freeze-fracture and transmission electron microscopic study. Am J Oto **4**, 214-221.

Saijo S, Kimura RS (1984) Distribution of HRP in the inner ear after injection into the middle ear cavity. Acta Otolaryngol (Stockh) **97**, 593-610.

Sakagami M, Tomiyama S, Fukazawa K, Umemoto M, Matsunaga T (1991) Capillary permeability of the stria vascularis in experimental hydrops: comparison between Kimura's model and immunologically induced model. J Clin Electron Microscopy **24**, 5-6.

Schuknecht HF (1976) Pathophysiology of endolymphatic hydrops. Arch Oto-Rhino-Laryng **212**, 253-262.

Schuknecht HF, Gulya AJ (1983) Endolymphatic hydrops. An overview and classification. Ann Otol Rhinol Laryngol **92**, Suppl **106**, 1-20.

Shambaugh GE, Roberts WS (1942) Chronic progressive deafness, including otosclerosis and diseases of the inner ear. Arch otolaryngol. **35**, 623-630.

Stahle J (1976) Allergy, immunology, psychosomatic, hypo- and hypertonus. Arch Otorhinolaryngol **212**, 287-292.

Suh KW, Cody DTR (1974) Obliteration of

vestibular and cochlear aqueduct in the guinea pig. Laryngoscope **4**, 1352-1368.

Takahashi M, Harris JP (1988) Anatomic distribution and localization of immuno-competent cells in normal mouse endolymphatic sac. Acta Otolaryngol (Stockh) **106**, 409-416.

Takahashi M, Tomiyama S (1990) Immunohistochemical study of endolymphatic hydrops due to secondary endolymphatic sac immune response. J Otolaryng Jpn **93**, 1372-1378.

Terayama S, Sasaki Y (1964) Studies on experimental allergic(isoimmune) labyrinthitis in guinea pigs. Acta Otolaryng **96**, 49-64.

Tomiyama S (1992a) Development of endolymphatic hydrops following immune response in the endolymphatic sac of the guinea pig. Acta Otolaryngol (Stockh) **112**, 470-478.

Tomiyama S (1992b) Endolymphatic hydrops induced by immune response of the endolymphatic sac: Relation to perilymph antibody levels. Ann Otol Rhinol Laryngol **101**, Suppl **157**, 48-53.

Tomiyama S, Harris JP (1986) The endolymphatic sac: its importance in inner ear immune responses. Laryngoscope **96**, 685-691.

Tomiyama S, Harris JP (1989) Elevation of inner ear antibody levels following direct antigen challenge of the endolymphatic sac. Acta Otolaryngol (Stockh) **107**, 202-209.

Tomiyama S, Nonaka M, Yagi T, Gotoh Y, Ikezono T (1991a) Development of acute endolymphatic hydrops following secondary endolymphatic sac immune response. J Otolaryng Jpn. **94**, 333-342.

Tomiyama S, Nonaka M, Yagi T (1991b) Caloric response and its histopathology following secondary immune response in the endolymphatic sac. Otol Jpn. **1**, 28-35.

Tomiyama S, Nonaka M, Yagi T (993) Vestibular immune injury following immune reaction of the endolymphatic sac. ORL, in press.

Wackym PA, Friberg U, Linthicum FH, Bagger-Sjoback D, Bui HT, Hofman F, Rask-Andersen H (1987) Human endolymphatic sac: Morphologic evidence of immunologic function. Ann Otol Rhinol Laryngol **96**, 276-81.

Woolf NK, Harris JP (1986) Cochlear pathophysiology associated with inner ear immune responses. Acta Otolaryngol (Stockh) **102**, 353-364.

Yoo TJ, Stuart JM, Kang AH, Townes AS, Tomoda K, Dixit S (1982) Type II collagen autoimmunity in otosclerosis and Meniere's disease. Science **217**, 1153-1155.

Yoo TJ, Yazawa Y, Tomoda K, Floyd R (1983a) Type II collagen-induced autoimmune endolymphatic hydrops in guinea pig. Science **222**, 65-67.

Yoo TJ, Tomoda K, Stuart JM, Cremer MA, Townes AS, Kang AH (1983b) Type II collagen-induced autoimmune sensorineural hearing loss and vestibular dysfunction in rats. Ann Otol Rhinol Laryngol **92**, 267-271.

Discussion with Reviewers

A.N. Salt: In the **Introduction** it is stated that the "endolymphatic sac is the only site in the inner ear in which immuno-competent cells ... can be found". However, in the **Discussion**, it is stated that the perilymphatically applied antigen induced hydrops and an immune reaction, apparently more severe than in the endolymphatic sac. Administration of antigen to the sac may give more consistent results but it is not true to say these agents only act at the sac.

Authors: It is generally accepted that immuno-competent cells cannot be seen in the normal inner ear except in the endolymphatic sac. This fact suggests an important role of the endolymphatic sac in the inner ear immune response, but does not imply that cellular infiltration into the inner ear is always derived from the endolymphatic sac. In fact, antigen challenge to the cochlea of immunized animals demonstrated severely deteriorative immune reaction in the cochlea which was accompanied with a profound cellular infiltration of immuno-competent cells from the spiral modiolar vein. This finding indicates that immuno-competent cells are locally supplied directly from the venules of the injection site, but not from the endolymphatic sac. However, immune reaction following antigen challenge to the cochlea of immunized animals was significantly reduced by obliteration of the endolymphatic sac. This result suggests that the endolymphatic sac must play a central role in the inner ear immune response.

In the case of antigen challenge to the endolymphatic sac of immunized animals, numerous immuno-competent cells infiltrated consistently and mainly into the endolymphatic sac which persisted for several months. On the other hand, very small number of immuno-competent cells occasionally infiltrated the saccule and the cochlea for a few days after challenge. These facts indicate that hydrops seen in this experiment is induced by a reaction of immuno-competent cells in the endolymphatic sac, but not by a reaction in the vestibule or cochlea.

A.N. Salt: It is surprising that a control group (antigen injection into the sac of non-immunized animals) is not included. This is especially important for the basal turn findings where hydrops is observed in as little as two hours after injection.

Authors: Previously (Tomiyama, 1992a), in control groups (primary injection with KLH into the sac and phosphate buffered saline injection into the sac), we were unable to induce endolymphatic hydrops one day after injection and later too. Subsequently, we concluded that endolymphatic hydrops seen in this experiment model was specifically induced by secondary immune reaction in the endolymphatic sac. In the present study, mild endolymphatic hydrops of the basal turn was seen in 2 out of 7 animals even as early as 2 hours after secondary challenge with KLH to the sac. These two animals already demonstrated moderate inflammatory

reaction in the sac, grade III. On the other hand, five animals with no hydrops formation showed mild inflammatory reaction in the sac. These results suggest that hydrops formation as early as 2 hours after injection must be induced by immune reaction in the sac rather than a non-specific effect of antigen injection to the endolymphatic sac.

R.S. Kimura: In methodology, 5 μl of KLH was injected into the endolymphatic sac. Is this amount not too large to inject into the endolymphatic sac of the guinea pig? According to our data, the sac volume is about 0.1 μl based on results from celloidin serial sections.

Authors: In this experiment, the bulged distal portion of the endolymphatic sac was observed after injection of KLH into the sac, suggesting a possibility of distention of the endolymphatic sac more than 0.1 μl. However, leakage of KLH occurred occasionally during injection into the endolymphatic sac. Leakage of KLH after injection may also occur, though the injection site was sealed with gelfoam to prevent leakage. To ascertain whether or not some amount of intradurally leaked KLH could cause endolymphatic hydrops, KLH challenge to the intradural space of immunized animal was performed. This did not result in formation of endolymphatic hydrops. To avoid a non-specific effect of a large amount of antigen injection into the endolymphatic sac on hydrops formation as pointed by the reviewer, further study should be carried out by injection of a very small amount of highly concentrated antigen.

H. Rask-Andersen: One may always question the possibility to inject such a large quantity as 5 μl in the endolymphatic sac without creating a kind of recirculation of the similar amount of fluid. Does this depend on the fact that some of these materials has been injected in the wall of the endolymphatic sac instead of intraluminary?

Authors: Injection of some amount of KLH into the endolymphatic sac wall cannot be surely ruled out, since KLH antigen was injected into the endolymphatic sac via the dura. To confirm whether or not injection of KLH antigen was correctly carried out into the lumen of the endolymphatic sac, any spread of KLH antigen just after injection was immuno-histochemically verified in our previous study (Takahashi and Tomiyama, 1990). KLH antigen was seen in the lumen of the endolymphatic sac, but not in the perisaccular tissue or in the perilymphatic region beyond the endolymphatic sac. These facts indicate that the majority of KLH antigen was successfully injected into the lumen of the endolymphatic sac.

R.S. Kimura: Is the endolymphatic hydrops shown in your specimens due to a widely spread immunological reaction, instead of interference of fluid drainage at the endolymphatic sac due to the localized inflammatory reaction?

Authors: In the early phase of this experiment, both a wide spread immunological reaction and an interfered function of the endolymphatic sac due to localized inflammatory reaction contributed to formation of endolymphatic hydrops. Our previous study (Takahashi and Tomiyama, 1990) demonstrated a significant spread of immuno-products (antigen specific IgG and C_3) in the cochlea which did not accompany the cellular infiltration. These facts suggest that chemical mediators released from the endolymphatic sac can widely spread to the inner ear in which an increased vascular permeability may occur. In fact, a significantly increased leakage of horseradish peroxidase from the stria vascularis and a significant elevation of serum antibody marker in perilymph on day 2 supported this speculation.

In the late phase, the present study suggested an interference of fluid drainage in the endolymphatic sac due to the prolonged localized inflammatory reaction.