Ha-Sheng Li
Malou Hultcrantz

* Department of Physiology II, Karolinska Institute;
  † Department of Otorhinolaryngology,
  Karolinska Hospital, Stockholm, Sweden

Key Words
Organ of Corti
Degeneration
Morphology
Electron microscopy
Mice
Genotype

Abstract
Degenerative changes in the organ of Corti were investigated in two genotypes of mice, CBA/Ca (moderate age-related late-onset hearing loss) and C57BL/6J (spontaneous early-onset auditory deterioration), ranging in age from 1 to 21 months (C57BL) and from 1 to 33 months (CBA). Light, transmission and scanning electron microscopes were used for qualitative and quantitative analyses. Alterations of deterioration in the CBA-mouse cochlea showed a late-onset loss of hair cells, lipofuscin in the outer hair cells (OHCs), giant stereocilia on the inner hair cells and elevated pillar heads. In C57BL mice, the primary lesions of the organ of Corti include early damage of stereocilia on OHCs, missing OHCs, and a ruptured reticular lamina. Giant cilia and elevated pillar heads were not evident in C57BL mice. In the aged C57BL mouse cochlea, the whole organ of Corti was collapsed and replaced by the supporting cells, whilst in the oldest CBA mice, completely normal-looking OHCs still remained even in severely deteriorated areas of the organ of Corti. The results indicate that the patterns of degeneration of the organ of Corti are different between CBA/Ca and C57BL/6J mice, probably under the control of different genes.

Introduction

Hearing loss associated with increased chronological age is referred to as presbyacusis. The basic etiologic causes remain unknown. Age-related hearing loss is believed to be due partially to a genetically programmed biological degeneration of the auditory system, and partially to the effects of a lifetime of exposure to both normal environmental and hazardous noise [1].

Schuknecht [2, 3] defined four types of presbyacusis based on the study of audiometric data and the histological findings in the temporal bones of aged humans: (1) sensory; (2) neural; (3) stria, and (4) cochlear conduc-
tive presbyacusis. These types may occur as a single element or in combination with each other. In humans, the pathological changes caused by the genetically modulated aging effect cannot be clearly distinguished from those that result from the inevitable exposure to environmental factors (noise, ototoxic agents, infections). The impact of hereditary factors on late-onset auditory degeneration is difficult to study in humans and, as a consequence, basic research with suitable animal models is needed.

The physiological correlates of aging and age-related sensorineural hearing loss have been studied in two inbred mouse genotypes, CBA and C57BL [4–6]. Age-related hearing loss in these two strains of mice is similar.
to that in humans in that auditory degeneration begins at the high frequencies, i.e., in the basal coil of the cochlea. C57BL mice demonstrate a progressive auditory decline with relatively early onset. The pattern of hearing loss in the C57BL strain closely resembles certain types of hereditary hearing loss observed in humans [7, 8]. CBA mice, often used as a control of C57BL mice, display a moderate auditory impairment with onset late in life. A comparison of the temporal aspects of hearing loss between the two genotypes of mice prompted us to investigate their susceptibility to environmental insults in strictly controlled conditions. Our recent reports suggested that the aging process is more precipitous in C57BL/6J mice than in CBA/Ca mice [9], and that C57BL mice are more susceptible to noise trauma than the age-matched CBA mice [10, 11]. The basic ultrastructural difference in degeneration of the cochlea between the two genotypes of mice is not well documented. Some studies on the aging cochlea in the two strains of mice have been mainly focused on the counting of hair cells by using the cytococheleogram technique, and a poor correlation between hair-cell counts with both behavioral and electrophysiological auditory measures has been reported [4, 5].

The hair-cell loss by itself is far too simple a measure to accurately predict the degree of age-related hearing loss, since the physiology of the remaining hair cells and their interactions with other cochlear structures are not known. The methods of the cytococheleogram, focusing mainly on hair-cell presence or absence, may have overlooked more subtle injuries to the surviving cells and surrounding tissues. The purpose of the present study was to investigate ultrastructural alterations of the aging organ of Corti and to compare the different patterns of the degeneration between CBA/Ca and C57BL/6J mice.

**Methods**

**Animals**

Two inbred strains of mice, a total of 20 CBA/Ca (Alab, Stockholm, Sweden) and 25 C57BL/6J (Department of Immunology, Karolinska Institute, Stockholm, Sweden), were used, ranging in age from 1 to 21 months (C57BL: 1, 2, 6, 7, 9, 12, 16, and 21 months) and 1 to 33 months (CBA: 1, 2, 6, 12, 24, and 33 months). At least 3 animals in each age group were studied. The median life span is about 31 months for CBA/Ca mice and 27 months for C57BL/6J mice, respectively [12]. The mice were kept at an ambient noise level of 30 dB(A), at room temperature (23–24°C) and in a 12 h light/dark cycle. Only animals with clearly normal tympanic membranes, as evidenced by otoscopic examination, were used. The animals, all females, were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and decapitated. The temporal bones were removed and immersed in Karnovsky's fixative (2% glutaraldehyde in cacodylate buffer pH 7.4) for further preparations for light microscopy (LM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM), according to standard methods [13].

**Preparations for LM and TEM**

One of the temporal bones from each animal was prepared for LM and TEM. The specimens were postfixed in 1% osmium tetroxide. After rinse in Ringer's solution and dehydration in increasing concentrations of alcohol, the cochleae were embedded in Epon mixture. Semithin sections for LM were stained with toluidine blue and ultrathin sections for TEM were stained with uranyl acetate and lead citrate. Light microscopical analysis was performed in a Zeiss Axioshot microscope and ultrastructural analysis was performed in a Philips 400 electron microscope.

**Preparations for SEM**

The opposite temporal bone from each animal was prepared for SEM. The cochleae were dissected (with removal of the bony parts and the tectorial membrane) in 70% alcohol. The critical-point method was used (Balzer's critical-point dryer 010). The specimens were sputter-coated with gold according to standard methods [13]. The results were analysed in a Philips 505 scanning electron microscope.

**Quantification**

Quantification in a four graded scale of surface pathology, as viewed with SEM, was performed using the cytococheleogram technique. The method used was based on the work of Hultcrantz et al. [13]. At least 3 cochleae from each age group in both strains of mice were analysed. From the middle coil, different rows of the outer hair cells (OHCs) were investigated and the normal, damaged or missing OHCs were counted. The surface pathology was classified in four different groups: normal, mildly damaged, severely damaged, and missing OHCs.

**Results**

**LM and TEM**

**CBA/Ca Mice.** The organ of Corti from 1 to 6 months of age showed normal structure. With increasing age, some OHCs were missing in the basal turn. In 33-month-old CBA mice (fig. 1a, b), most OHCs degenerated, the number of the mitochondria was reduced, and lipofuscin was found in OHCs. Some cuticular plates still remained. Giant stereocilia were found, but only on the inner hair cells (IHCs). The density of the myelinated cochlear nerve fibers and spiral ganglion cells was reduced.

**C57BL/6J Mice.** At 1 and 2 months of age, some OHCs were damaged. A few dilated vesicles were observed in the base of IHCs. With increasing age, OHC damage became evident. In 16-month-old mice, a severe loss of OHCs was replaced by the supporting cells (Deiters' cells). At 21 months of age, the whole organ of Corti was collapsed in the basa lturn and only a few spiral ganglion cells remained.
**Fig. 1.** CBA mice (33-month-old): Middle turn. **a** LM. A giant stereocilium on IHC (arrow) and degeneration of OHCs (asterisk). Note a reduced density of the nerve fibers (nf). × 270. **b** TEM. Arrowheads indicate lipofuscin in OHCs. Cuticular plate still remained (arrow). × 5,300.

**Fig. 2.** SEM. CBA mice (33-month-old): Middle turn. **a** Missing OHCs (asterisk), elevated pillar heads (arrowhead) and a sunken reticular lamina. A few normal-looking OHCs remaining and protruding in severely degenerated areas. Giant stereocilia on IHCs (arrow). **b** A giant hair on IHC bent towards the modiolus. Bar = 1 μm.

**SEM**

**CBA/Ca Mice.** SEM shows a normal morphology of the organ of Corti up to 12 months of age. In 24-month-old CBA mice, missing OHCs and a few elevated pillar heads in the middle coil were observed. Remaining OHCs showed an almost normal appearance. A slight disarray of sensory hairs on IHCs was seen. At 33 months of age (fig. 2a) a pronounced degeneration was observed, including missing OHCs and elevated pillar heads. Even so, some normal-looking OHCs still remained in severely degenerated areas and protruded over the reticular lamina. Stereocilia on IHCs were fused, disarrayed, blebbled and missing. Giant sensory hairs on IHCs were frequent in the middle and apical coils and, interestingly, bent towards the modiolus (fig. 2b).

**C57BL/6J Mice.** In 2-month-old C57BL mice, a few OHCs were missing but IHCs were normal in the basal coil of the organ of Corti. At 7 months of age, severely degenerate OHCs were observed with fused, disarrayed, inclined, and missing sensory hairs. The upper part of
OHCs shrunk and reticular lamina ruptured (fig. 3a). Some damaged OHCs protruded over the reticular lamina with disrupted cuticular plates (Fig. 3b). However, the hairs on IHCs still remained normal looking and extra IHCs were observed (fig. 3a). In 12-month-old C57BL mice, a severe loss of OHCs and a few remaining OHCs with severely damaged stereocilia were observed (fig. 4a, b). Some sensory hairs on IHCs were missing or fused. The basal coil of the organ of Corti in 16-month-old mice was devoid of OHCs and scar formation was noted in these regions. Few IHCs remained in the barren area. In the aged C57BL mouse cochlea, elevated pillar heads and giant cilia were not evident.

**Quantitative Findings**

Counting of degenerated sensory hairs and missing OHCs from the middle coil of the cochlea was done according to a four-graded scale. Figures 5a, b show the
aging process of OHCs in CBA and C57BL mice, respectively. The CBA mouse cochlea showed a degeneration pattern with either remaining relatively normal OHCs or missing OHCs, but few severely damaged cells. By contrast, progressive deterioration of stereocilia with all variations of damaged OHCs was observed in the C57BL mouse cochlea and accelerated with increasing age.

**Discussion**

The two strains of mice show different susceptibility to age-related hearing loss [4–6, 9]. The mean ABR thresholds increased with age, first at the high frequencies and more rapidly and severely for C57BL mice than CBA mice. The present study demonstrated different patterns of degeneration in the organ of Corti between the two strains of mice. The major pathological alterations in CBA/Ca mice showed a late-onset loss of OHCs, lipofuscin in OHCs, elevated pillar heads, and giant stereocilia on IHCs. The most striking features in C57BL/6J mice were an early-onset progressive degeneration of stereocilia, a severe loss of OHCs, a broken reticular lamina, and scar formation.

In our study, TEM showed a less specific difference in the aging organ of Corti between the two strains of mice than SEM. It was reported that the most striking and common ultrastructural changes in aging guinea pigs was the degeneration of the cuticular plate [14]. Our study showed that hair-cell body degenerated first, followed by the cuticular plate. Lipofuscin is one of the most common signs of aging in the cochlear and vestibular sensory epithelia [15, 16]. In our study lipofuscin was observed in OHCs of the aged CBA-mouse cochlea (33 months old). The lipofuscin seems to be a linear marker of time passing and has no cytotoxic effect and no relationship with any of the dementing disorders [17].

The present study showed that OHCs were more susceptible to aging effects than IHCs for both strains of mice. The findings are also in agreement with human studies [18]. Acoustic and ototoxic insults also typically cause greater damage to OHCs than IHCs [19, 20]. One possible explanation of the difference in susceptibility between OHCs and IHCs is based on a number of morphological differences in the organ of Corti [21]. These include differences in the intracellular organization, the cells’ size and shape, the organization of their stereocilia, the relationship with tectorial membrane synaptic structures, as well as their surroundings. It is believed that IHC sensory hairs are free standing, whereas the tallest rows of OHC sensory hairs are attached to the tectorial membrane [22]. The extracellular membrane of the OHC is, in part surrounded by perilymph, whereas the IHC is surrounded by supporting cells. Consequently, OHCs would be more vulnerable to mechanical stress, toxic effects or metabolic exhaustion than IHCs.
The elevated pillar heads in the aged CBA mouse cochlea formed a striking contrast to that observed in C57BL mice in which the elevated pillar heads were rarely seen. The findings on the role of the supporting cells in preventing severe nerve degeneration have been reported by Bredberg [18] and Johnsson and Hawkins [23]. Our result implies a healing capacity of the pillar cells in CBA mice. Interestingly, some normal-looking OHCs in old CBA mice still remained in severely degenerated areas. This observation indicates that OHCs in CBA mice resist the aging effects. However, in C57BL mouse cochlea, including young adults, extensively damaged stereocilia were observed on a large number of preserved OHCs. The results suggest that stereocilia are the main part of the OHCs prone to deterioration. We do not know what cellular structure of OHCs initiates the degeneration, the cell body or the stereocilia. A collapsed organ of Corti devoid of hair cells and scar formations were frequent findings in the aged C57BL mouse cochlea. A ruptured reticular lamina in C57BL mice may be due to a shrinking of the upper part of OHCs. Since the cell body of the OHC is bathed in perilymph, changes of the reticular lamina would bring potassium-rich endolymph into contact with OHCs, and would represent a toxic environment for OHCs.

Another significant finding in the aged CBA mice was the appearance of giant stereocilia on IHCs. OHCs were not present with this change. The process includes disarray, fusion, expansion or elongation of groups of stereocilia. Such changes have also been observed in the human cochlea [24–27]. From the human data, giant stereocilia are represented on both IHCs and OHCs except the OHCs in the basal coil. In our study, the giant stereocilia were present only on IHCs in the middle and apical coils of the CBA mouse cochlea. The reason may be that the sensory hairs of the basal coil are normally shorter and may not be as susceptible to disarray. The typical changes of the giant stereocilia on IHCs in the middle coil of the aged CBA mouse cochlea were not found in C57BL mice. The reason might be that the life span of these cells in C57BL mice is too short to have time forming the giant cilia.

The hair cell damage accompanied by the giant stereocilia on IHCs in CBA mice is also observed in noise exposed animals [28–30]. It has been suggested that the differences between species with respect of IHC damage are related to the degree of development of Hensen’s stripe on the lower surface of the tectorial membrane and its contact with the IHC cilia [28]. On the other hand, the giant cilia possibly indicate a regenerative activation of the cells [30].

In C57BL mice, disarrayed and fused stereocilia on OHCs were observed at the early degeneration stage. There is a structural cross-link between the tips of the stereocilia and adjacent taller stereocilia rows. When the stereocilia were not in disarray, the tip links remained intact [31]. Otherwise, these cross-links could be impaired, which would alter the bundle properties of the stereocilia and directly affect hair-cell function. The filaments of actin are densely packed within the stereocilia as well as the cuticular plates of the hair cells. The formation of actin seems to be a vulnerable point in the hair cell metabolism and could be affected by genetic factors, aging and environmental insults. Phalloidin-labelled actin expressions in the cuticular plate confirm the degeneration differences between the two strains of mice [32]. The actin genes could be coded in the very genome of the hair cell nucleus. The response to injury is governed by both the heritable genetic state and the somatic genetic changes accumulated during the life of the organism. Intracellular organelle regeneration is largely dependent on effective DNA repair [33]. The inability of DNA to repair efficiently as well as mistakes in other control mechanisms would result in a disorganization of the cell structure. This situation could be seen pathologically in hair cells as distorted cytoskeletal structures. It may result in altered architecture, with reduced function. The susceptibility and the resistance to age-related auditory degeneration are individually determined, with important environmental as well as genetic contributions. What causes the expression of genes that results in the observed effects of aging? Increased knowledge of the molecular genetics has aided in the investigation of the mechanisms of aging. It has been recently reported that age-related hearing loss is produced by a small number of recessive genes, one of which produces age-related hearing loss in C57BL mice [34].

Acknowledgements

The authors wish to thank Professor E. Borg for valuable discussions and Mrs. W. Ring and Mrs. M.L. Spångberg for their technical assistance. This work was supported by Medical Research Council grant (0072), Swedish Work Environment Found, Foundation Tysta Skolan, Wenner-Gren Center Foundation, Törnstrand Fund and Karolinska Institute Funds.

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