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Cytomegalovirus-induced pathology in human temporal bones with congenital and acquired infection

Vladimir Tsuprun[a,*], Nevra Keskin[a,b], Mark R. Schleiss[c], Pat Schachern[a], Sebahattin Cureoglu[a]

[a] Department of Otolaryngology-Head and Neck Surgery, University of Minnesota, Minneapolis, MN, USA
[b] Department of Veterinary Internal Medicine, Ankara University, Ankara, Turkey
[c] Division of Pediatric Infectious Disease and Immunology, Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA

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ABSTRACT

Objective: Publications on histopathology of human temporal bones with cytomegalovirus (CMV) infection are limited. We aim to determine histopathology of the inner ears and the middle ears in human temporal bones with congenital and acquired CMV infections.

Methods: Temporal bones from 2 infants with congenital and 2 adults with acquired CMV infection were evaluated by light microscopy.

Results: Two infants with congenital CMV infection showed striking pathological changes in the inner ear. There was a hypervascularization of the stria vascularis in the cochlea of the first infant, but no obvious loss of outer and inner hair cells was seen in the organ of Corti. However, cytomegalic cells and a loss of outer hair cells were found in the cochlea of the second infant. The vestibular organs of both infants showed cytomegalic cells, mostly located on dark cells. There was a loss of type I and type II hair cells in the macula of the saccule and utricle. Loss of hair cells and degeneration of nerve fibers was also seen in the semicircular canals. Both infants with congenital infection showed abundant inflammatory cells and fibrous structures in the middle ear cavity. No evidence of cytomegalic cells and hair cell loss was found in the cochlea or vestibular labyrinth in acquired CMV infection.

Conclusions: In two infants with congenital CMV infection, the cochlea, vestibule, and middle ear were highly affected. Temporal bones of adult donors with acquired viral infection showed histological findings similar to donors of the same age without ear disease.

1. Introduction

Sensorineural hearing loss is one of the most common complications associated with cytomegalovirus (CMV) infection. Between 10 and 20% of infants congenitally infected with CMV have varying degrees of hearing loss, which can be present at birth or may manifest in the first months and years of life [1–4]. Vestibular impairment is also frequent, and under-recognized [5,6]. CMV can be transmitted to the fetus either in the setting of re-infection or reactivation of latent infection in women with preconception immunity, or in the setting of primary infection during pregnancy [7]. The mechanism of hearing loss caused by CMV infection is poorly understood, but has been explored in animal models of infection, including neonatal mice [8,9] and congenitally infected guinea pigs [10]. In the guinea pig model of congenital CMV infection, the guinea pig CMV has been shown to cross the placenta, directly infecting the pup *in utero*, and results in various congenital disorders in newborns, including hearing loss [11,12]. Direct injection of CMV into the inner ears of adult guinea pigs also resulted in histopathological changes and sensorineural hearing loss [13,14]. It has been proposed that inner ear injury leading to hearing loss in the guinea pig model may be mediated, at least in part, through expression of a virally-encoded cytokine, macrophage inflammatory protein (MIP) [15,16]. Histological evaluation of inner ears in the guinea pig model has demonstrated damage of hair cells and loss of spiral ganglion neurons. Viral antigens have been found in the organs of Corti, spiral ganglion, scala media, and Reissner's membrane [17,18]. In the guinea pig vestibular system, the utricle and saccule were atrophied, but no CMV infection was found [19–21]. Immunohistochemistry or PCR analysis can be used for identification of CMV; however, in many tissues with high loads of the virus, the presence of virus has been inferred by

* Corresponding author at: Otopathology Laboratory, Department of Otolaryngology, University of Minnesota, Room 226, 2001 6th St. SE, Minneapolis, MN 55455, USA.
E-mail address: tsupr001@umn.edu (V. Tsuprun).

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identification of cytomegalic cells containing characteristic inclusion bodies [22].

There have been limited descriptions of the cochleovestibular pathology of congenital and acquired CMV infections in humans. Analyses of human temporal bones of infants and fetuses has revealed cytomegalic cells with inclusion bodies in the cochlea and vestibular labyrinth, degeneration of organs of Corti and spiral ganglion cells in the basal cochlear turns, atrophy of the crista and degeneration of nerve fibers in the vestibular system [23–25]. In study of the inner ear of an infant with severe congenital CMV infection that utilized immunofluorescent and electron microscopy, Davis et al. [26] found injury in the first half of basal turn of the cochlea. The authors demonstrated severe damage of the saccule and utricle in vestibular organs and cytomegalic cells concentrated in the regions of dark cells in the utricle. CMV has also been detected in the perilymph of the human inner ear [27–29]. Studies of fetuses at 21-weeks gestational age revealed evidence of viral infection in the marginal cell layer of the stria vascularis, Reissner's membrane, and the sensory cells in the utricle and in the crista ampullaris [30]. A temporal bone of a child with acquired CMV infection demonstrated that the epithelium of the endolymphatic sac, the utricle, and the semicircular canals contained cytomegalic cells; there was loss of inner and outer hair cells, and of cochlear ganglion cells [31]. However, the fact that the infant reported in this case had been treated with gentamicin did complicate the histopathological analysis. In contrast, most immunocompetent adults infected with CMV show no clinical symptoms, and no convincing evidence for post-natal acquisition of infection leading to hearing loss. Temporal bones obtained postmortem from HIV-positive adults with evidence of active CMV disease demonstrate that cytomegalic cells were found in the middle and inner ears in 6 (24%) of 25 patients [32].

The objective of this study was to evaluate temporal bone specimens in infants with a documented history of congenital CMV infection for auditory and vestibular histopathology, and to compare these findings with those observed in adults with acquired CMV infections, toward the goal of better understanding the pathophysiology of hearing loss and vestibular dysfunction in CMV-infected patients.

2. Materials and methods

Human temporal bones from two deceased newborn infants with congenital CMV infection (Cases 1 and 2) and from two deceased adult donors with acquired virus infection (Cases 3 and 4) were identified in the Otopathology laboratory at the University of Minnesota, Minneapolis, MN and the Massachusetts Eye and Ear Infirmary, Boston, MA.

The temporal bones were removed after death. The postmortem time at autopsy varied between 6 and 14 h. Damage to temporal bone structures due to autolysis appeared to be limited. Temporal bones were fixed in formalin solution, decalcified in ethylenediaminetetraacetic acid (EDTA) and embedded in celloidin. Serial sectioning was performed in the horizontal plane at a thickness of 20 µm. Every tenth section was stained with hematoxylin and eosin (H&E) and mounted on glass slides for light microscopic observation. To evaluate some types of hair cells and other cells, we also used a differential interference contrast (DIC) microscopy. The Institutional Review Board of the University of Minnesota approved this study (0206 M26181).

Middle and inner ear of temporal bones with CMV infection were compared to age-matched temporal bones of patients with no history or histological signs of ear disease. Two of the authors were responsible for blinded analysis of the temporal bones. We characterized histological changes in the basilar, middle, and apical cochlear turns for loss of hair cells and neurofilaments, abnormalities of the stria vascularis and spiral ligament, and for the presence of cytomegalic cells with CMV inclusion bodies. In the peripheral vestibular system, we analyzed the loss of vestibular hair cells, degeneration of vestibular nerves, and presence of cytomegalic cells. In the middle ear, presence of inflammatory cells was noted. Both adult donors with acquired CMV infection had leukemia; we compared these cases to temporal bones of age-matched donors without CMV infection and leukemia; there were 9 temporal bones of adult donors without CMV infection who had leukemia in our collection.

Clinical histories:

Case 1. This 11-day-old full-term old boy was noted to be icteric, and was found to have an enlarged liver and spleen and a generalized petechial rash. In spite of a total volume exchange transfusion, bilirubin levels continued to rise. After 6 days, hypothermia and anemia were noted. His clinical condition worsened with persistent elevation of liver enzymes, and he succumbed nine hours later. Autopsy findings revealed a widely disseminated CMV infection associated with necrotizing enterocolitis.

Case 2. A 3-week-old girl developed hepatomegaly when she was 2 days old and was immediately admitted to the hospital. She was diagnosed with erythroblastosis fetalis and treated for this disease. Two weeks later she developed exfoliative dermatitis and severe dehydration. She succumbed despite intensive treatment. Cytomegalic cells were seen in the renal tubules and many other organs at autopsy.

Case 3. This 37-year-old white female was diagnosed with chronic myelogenous leukemia. She underwent chemotherapy with hydroxyurea and an allogeneic bone morrow transplant was performed. The patient died due to multiorgan failure and adult respiratory distress syndrome. She had been diagnosed with CMV infection and cytomegalic cells were found on esophageal biopsy (CMV esophagitis). There was no history of hearing loss or vestibular problems in her clinical case record.

Case 4. A 31-year-old white female, 57 days post-bone marrow transplant for chronic myelogenous leukemia, developed mild shortness of breath. X-ray revealed new bilateral reticulonodular infiltrates and bronchoscopy was performed. The results disclosed diffuse interstitial CMV. Patient received a 10-day course of ganciclovir. There was no history of hearing loss or vestibular disorder in her clinical records.

3. Results

Temporal bones of newborn infants without CMV infection or other ear diseases have intact inner and outer hair cells, no atrophy of the stria vascularis, and no loss of fibrocytes in the spiral ligament (Fig. 1a). Analyses of temporal bones of two newborns with congenital CMV infection (Cases 1 and 2) from our temporal bone collection demonstrated several pathological changes in the cochlea (Fig. 1b, c). In Case 1 (Fig. 1b), hypervascularization and enlarged blood vessels were visible in the stria vascularis in all cochlear turns. There was also deterioration of the integrity of cells in the stria vascularis, with a loss of cellularity and the presence of edema, most notable in the intermediate layer. Apparently normal numbers of outer and inner hair cells in the organ of Corti were noted (Fig. 1b, inset). However, in Case 2, a loss of outer cochlear hair cells (Fig. 1c, arrow) was observed. To identify CMV in the inner ear, we evaluated the tissue for the presence of inclusion-bearing cytomegalic cells noted in hematoxylin-eosin stained sections of temporal bones. In Case 2, large “owls-eye” cytomegalic cells with CMV inclusion bodies were observed in the cochlear duct, including cells adjacent to Reissner's membrane (Fig. 1c, d). No cytomegalic cells were found in the organ of Corti.

The sensory epithelium in the vestibular system of congenitally infected newborn infants demonstrated a loss of type I and type II hair cells in the macula of the utricle (Fig. 2a) and saccule (Fig. 2b) compared to normal temporal bones (Fig. 2c). There was also a loss of vestibular hair cells and degeneration of vestibular nerves in the semicircular canals (Fig. 2d, e). Cytomegalic cells were mostly attached to the dark cells in the semicircular canals and utricle of Case 1 (Fig. 2d)

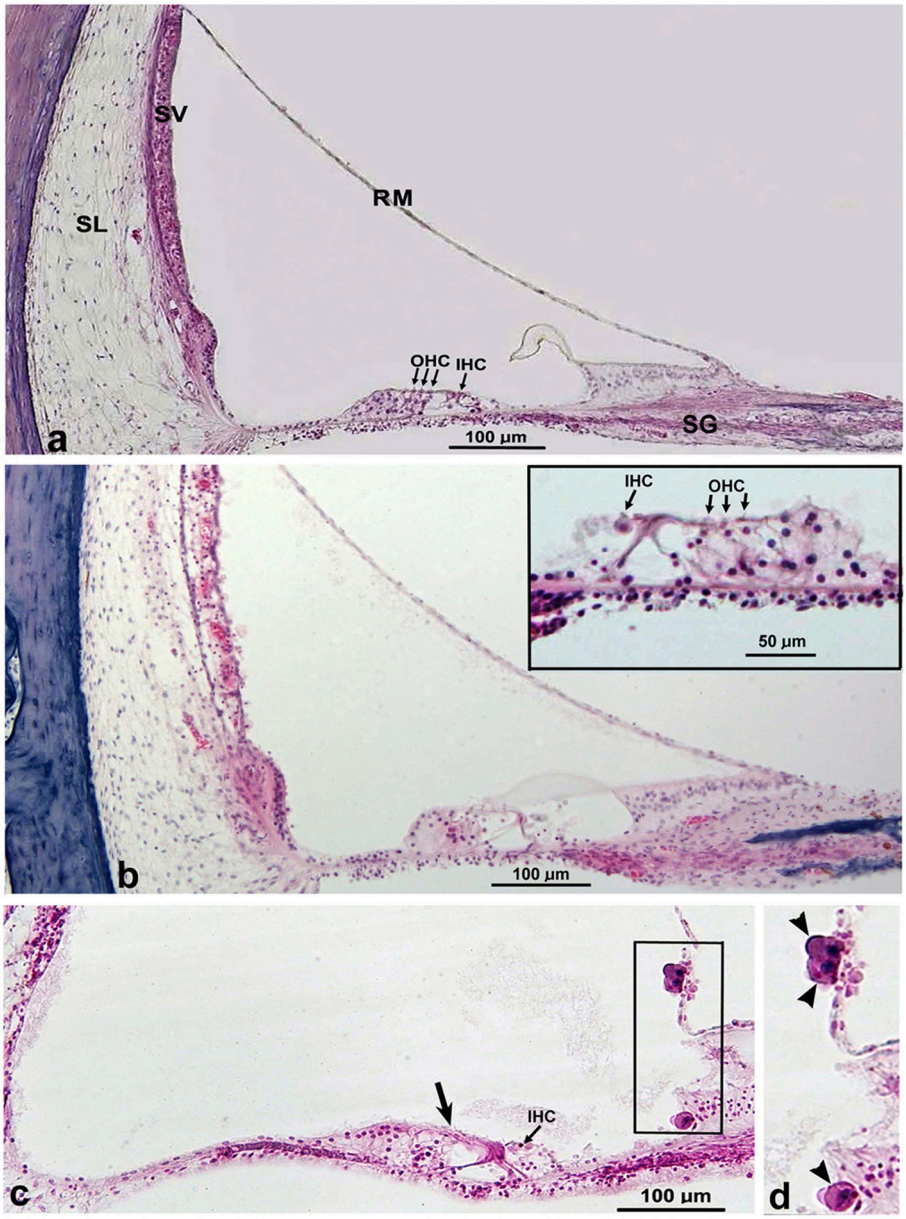

Fig. 1. a: Image of control temporal bone of a 2-day-old boy. b: Microscopic examination of a temporal bone of an 11-day-old boy with congenital CMV infection (Case 1) revealing hypervascularization and enlarged blood vessels in the stria vascularis, with the intermediate layer the most affected. The organ of Corti demonstrates outer and inner ear hair cells (b, inset). c: Temporal bone of a 3-week-old girl with congenital CMV infection (Case 2) with a notable loss of outer hair cells (arrow). d: Enlarged view of the boxed area in (c). Arrowheads indicate cytomegalic cells in the endolymphatic space of the cochlea. Hematoxylin and eosin staining. SV = stria vascularis, SL = spiral ligament, RM = Reissner's membrane, IHC = inner hair cells, OHC = outer hair cells, SG = spiral ganglion.

and Case 2 (Fig. 2e). Fewer cytomegalic cells were observed on the wall and in the epithelium of the vestibular labyrinth.

It was noted for Case 1 (Fig. 3a) and Case 2 (Fig. 3b), in these infants with congenital CMV infection, that the middle ear cavities were filled with the abundant inflammatory cells, both monocytes and neutrophils, as well as with fibrous structures (Fig. 3b, inset).

In contrast, temporal bones of adult donors (Cases 3 and 4) with acquired CMV infection (Fig. 4a, b) had minimal or no evidence of histopathology upon microscopic examination. Both outer and inner hair cells were noted (Fig. 4a, b). There was mild atrophy of the stria vascularis in the middle turn of the cochlea (Case 4) and loss of fibrocytes in the spiral ligament, more noticeable for type I and type II fibrocytes in both donors. Some melanin-like pigmentation was observed in the stria vascularis; however, we have previously seen similar granules in human temporal bones of donors without CMV infection (not shown), but, as in these patients, having chronic myelogenous leukemia and having undergone bone marrow transplantation. The sensory epithelium of the vestibular system did not demonstrate loss of hair cells or other structural abnormalities in either case of acquired CMV infection. No evidence of cytomegalic cells was found in the cochlea or vestibular membranous labyrinth. There were some inflammatory cells in the protympanum and along the Eustachian tube in Case 3.

Table 1 summarizes the prominent histopathological changes observed in the auditory and vestibular systems in the human temporal bones from individuals with congenital and acquired CMV infection that were examined in this study.

4. Discussion

We observed multiple pathological changes in the cochlea, vestibular organs (saccule, utricle, and semicircular canals), and in the middle ear in this examination of temporal bones acquired from newborn infants with congenital CMV infection. Cochlear changes, such as loss of outer hair cells and cells of the stria vascularis in these infants (Fig. 1b, c), are consistent with reported clinical records of pathology in infants and young children with sensorineural hearing loss associated with congenital CMV infection [1–4]. Cells of the stria vascularis are involved in maintenance of ion and fluid homeostasis in the cochlea. These cells are important for mechanoelectrical transduction of sound

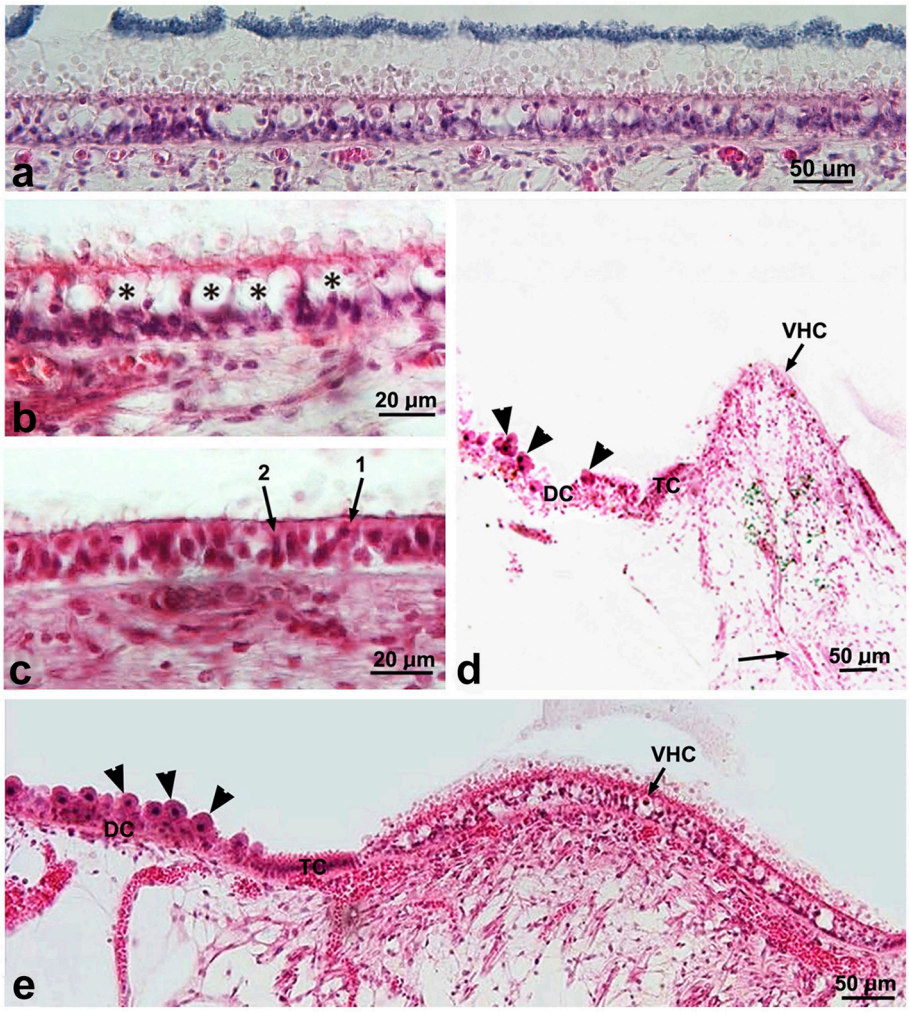

Fig. 2. a: Representative image of the maculae of utricle in a human temporal bone of infant with congenital CMV infection (Case 1) as seen by DIC microscopy. b: Loss of type I and type II vestibular hair cells (asterisks) in the maculae of the saccule (Case 1). c: Vestibular types I and type II hair cells (arrows) in normal temporal bone. d, e: Loss of vestibular hair cells and degeneration of vestibular nerves (arrow) in the posterior semicircular canal (Case 2) (d) and in the lateral semicircular canal (Case 1) (e). Cytomegalic cells (arrowheads) are located along the dark cells. Hematoxylin and eosin staining. VHC = vestibular hair cells. DC = dark cells. TC = transitional cells.

in hair cells and their loss, in turn, can contribute to hearing impairment [33].

In Case 1, other factors, such as an increased level of bilirubin, could have contributed to loss of hair cells and the other histopathological changes in the inner ear [34]. However, the pathophysiology of inner ear damage secondary to hyperbilirubinemia is not well-defined. Belal et al. [35] reported that the cochlea does not appear to be directly affected by hyperbilirubinemia. We therefore hypothesize that the changes we observed were more likely due to congenital CMV infection.

CMV is a virus from the *Herpesviridae* family that replicates in the nuclei of infected cells [36]. Cytomegalic cells with inclusion bodies have been reported in tissues with high viral load [22]. We found cytomegalic cells on Reissner's membrane and in other sites of the cochlear endolymph in newborn infants with congenital CMV infection; however, they were not observed in the organ of Corti. More cytomegalic cells were observed in the vestibular system of these infants than in the respective cochleas. These cells were mostly located along the dark cells in the semicircular canals and utricle. Some of the dark cells in contact with or in the vicinity of cytomegalic cells appeared to be damaged (Fig. 2d, e). Our data are consistent with a high concentration

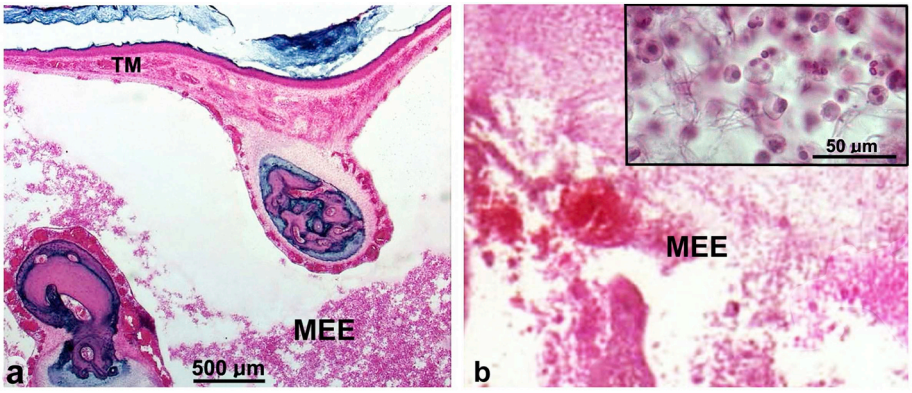

Fig. 3. Middle ear of newborn infants with congenital CMV infection in Case 1 (a) and Case 2 (b). Enlarged images demonstrate the presence of inflammatory cells and fibrous structures in the middle ear cavity (b, inset). Hematoxylin and eosin staining. MEE = middle ear effusion. TM = tympanic membrane.

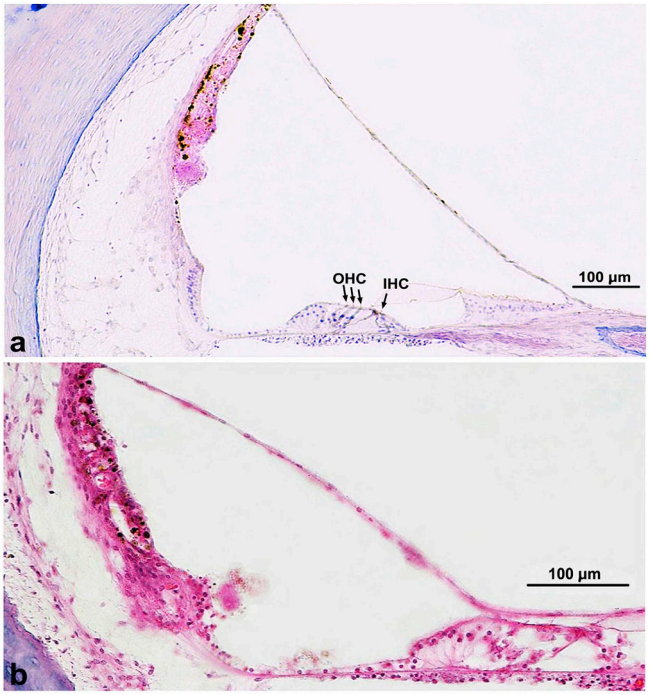

Fig. 4. Analysis of temporal bones of adults with acquired CMV infection in Case 3 (a) and Case 4 (b) demonstrates only mild pathological changes in the cochlea and no loss of outer or inner hair cells. Hematoxylin and eosin staining. IHC = inner hair cells, OHC = outer hair cells.

of cytomegalic cells in the regions of dark cells in the utricle as noted by Davis et al. [26]. The presence of cytomegalic cells may be related to a high viral load leading to loss of vestibular hair cells in the macula of the saccule and utricle, and to the loss of hair cells and/or degeneration of vestibular nerves in the semicircular canals. The vestibular hair cells are involved in mechanoelectrical transduction [37], while the dark cells are responsible for endolymph secretion [33,38]. Both types of cells and vestibular nerves were affected in temporal bones with congenital CMV infection, explaining the symptoms of balance disorders and dizziness that are frequent in these infants [6] who have partial and bilateral vestibular impairment in 43% [39] and balance disorders in 90% of cases [4]. In both Cases 1 and 2, cytomegalic cells were noted in the vestibular system. This may reflect a high viral load in the vestibular labyrinth.

Infants with congenital CMV (Cases 1 and 2) demonstrated more extensive damage to hair cells and other inner ear structures in areas containing cytomegalic cells, probably due to the direct cytopathic effect of the virus. Notably, there were some inflammatory cells in the spiral ganglion; however, the presence of inflammatory cells in the cochlear perilymph and endolymph was insignificant. A high dose of virus used in some experimental animal models produced a robust inflammatory response in the inner ear that could contribute to CMV-induced histopathology. We have previously reported that direct inoculation of CMV into perilymph *via* the round window membrane in guinea pigs resulted in a large number of inflammatory cells in the scala tympani and demonstrated a specific role of virally encoded chemokine MIP (microphage inflammatory protein) in inducing pathological changes and generating hearing loss associated with CMV [15,16]. In neonatal mice, intracerebral inoculation with CMV-induced loss of cochlear hair cells; macrophages and lymphocytes were preferentially immunolocalized close to virus particles in perilymphatic epithelium and spiral ganglion neurons [8]. Examination of neonatal mice inoculated intraperitoneally with CMV revelated viral antigens and CD3+ mononuclear cells in areas of the spiral ganglion cells and stria vascularis [18]. Thus, both our observations and correlative data from relevant animal models supports a key role for inflammation in the pathogenesis of CMV-induced inner ear pathology.

Interestingly, the temporal bones of infants with congenital CMV infection in both Cases 1 and 2 demonstrated evidence of abundant inflammatory cells in the middle ear cavity. Although inner ear pathology is generally accepted as the final arbiter of hearing loss in the congenitally infected infants, an association of CMV with otitis media has been previously reported by Chonmaitree et al. [40], who found that about 10% of infants and children with acute otitis media were also infected with CMV or herpes simplex virus type 1. Further evaluation of middle ear dysfunction associated with CMV infection is warranted in order to examine what additional role that infection of this compartment could play in hearing loss. Other pathology we noted includes hypervascularization and dilation of blood vessels in the stria vascularis as well as the peripheral vestibular system. CMV has been reported to induce cytokines and growth factors associated with angiogenesis [41] which could explain these findings, although the role of these pathologies in both acute symptomatic disease and long-term sequelae requires additional clarification.

Neither of the adult donors with acquired CMV infection (Cases 3 and 4) had a history of hearing loss or vestibular dysfunction. Their temporal bones revealed little or no pathology, consistent with reports that most immunocompetent children and adults infected with CMV do not develop clinical symptoms. Cochlear and vestibular hair cells of adult donors were intact, but we observed some melanin-like pigmentation in the stria vascularis. We have seen similar pigment granules in human temporal bones from age-matched adult donors with leukemia, but without CMV infection. Melanin-bearing cells of the stria vascularis have been reported to have increased activity in response to infection, noise, and harmful substances [42,43]. CMV-induced pneumonia and respiratory distress syndrome are common in CMV-infected oncology patients and associated with a high early mortality rate [44]. Two donors (Cases 3 and 4) had myelogenous leukemia and had undergone bone morrow transplantation. In our study, the adult patients had clinical histories of respiratory distress and one of those cases (Case 3) had inflammatory cells, mainly in the protympanum and along the Eustachian tube.

In summary, in this study, we observed congenital CMV infection in

Table 1
Histological findings in the temporal bones of newborn infants with congenital (Cases 1 and 2) and adult donors with acquired (Cases 3 and 4) cytomegalovirus infections.

Case	Age/Sex	Cochlea	Vestibular system	Middle ear
1	11 days/M	Hypervascularity, loss of cells in the stria vascularis No cytomegalic cells	Loss of vestibular hair cells Degeneration of nerves	Inflammatory cells, fibrous structures in the middle ear cavity
2	3 weeks/F	Loss of outer hair cells Cytomegalic cells	Cytomegalic cells	
3	37 years/F	No	No	No
4	31 years/F	No	No	No

newborn infants with prominent histopathological changes in the cochlea, vestibular system, and middle ear; however, no remarkable ear pathology was found in adults with acquired CMV infection. Congenital CMV infection is the leading infectious cause of brain damage and hearing loss in children and CMV is a relevant health issue to transplant recipients and HIV-infected patients [45]. Acquired CMV infection is usually asymptomatic in adults but can become symptomatic in patients with compromised immune systems [46]. Cytomegalic cells have been reported in the inner and middle ears of 12 human temporal bones from 25 adults (49 temporal bones) with acquired CMV and HIV co-infections [32]; however, we did not detect cytomegalic cells in the middle and inner ears of our temporal bones of adult donors with acquired CMV infection, possibly because the magnitude of immune suppression was greater in the HIV-infected subjects.

5. Conclusions

We noted CMV-associated histopathological changes that were more severe in the congenitally infected group that those noted in the acquired infection group. In infants with congenital infection, cytomegalic cells were most prominent in vestibular organs. The cochlea, vestibular system, and middle ear were all highly affected. These histopathologies provide a framework for further studies to investigate the pathogenesis of CMV-induced auditory and vestibular disorders in infants with congenital CMV infection.

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