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Measles Virus and Otosclerosis

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Abstract

Measles virus (MeV) might play an important role as an environmental stimulus in the etiopathogenesis of otosclerosis. Chronic inflammation was shown in morphologic investigations of otosclerotic foci and MeV N, P, and F proteins were detected within cells of the otosclerotic focus by immunohistochemical investigations. MeV RNA was extracted from fresh-frozen otosclerotic tissue by the use of in vitro RT-PCR. This result was validated through amplification of MeV genome sequences by RT-PCR from celloidin-embedded sections with morphologically ascertained otosclerotic foci. In searching for an immune response of the inner ear immune system against MeV proteins, elevated anti-MeV IgG levels were detected in the perilymph of patients with otosclerosis in comparison with the serum levels. In situ RT-PCR allowed the localization of MeV sequences in osteoclasts, osteoblasts, chondrocytes, macrophages, and epithelial cells in middle ear mucosa of otosclerotic tissue. Further evidence for MeV persistence has recently been given. Genotyping of MeV in otosclerotic foci demonstrated the presence of MeV genotype A, which circulated in Europe around 1960. All the above results confirm a strong association between MeV and otosclerosis.

Otosclerosis was described for the first time by Antonio Maria Valsalva in 1735 [1] as a disease of the human temporal bone. More than one century later, Toynbee [2] recognized otosclerosis as a cause of hearing loss. Otosclerosis may occur as a histological type within the human temporal bone without affecting the stapes footplate. In only 10% of patients with otosclerosis is the focus localized near the oval window niche leading to fixation of the stapes with consecutive (characteristic) conductive or mixed hearing loss. Women are affected 1.4 times more frequently than men and the age of onset has risen in Caucasians in the last decades [3]. Otosclerosis is the most important cause of hearing loss

in Europe and the USA, whereas it appears to be uncommon in developing countries and among the Japanese population [4].

Morphologic Analysis of the Otosclerotic Focus

Otosclerosis only affects the human temporal bone, but otosclerosis-like lesions were observed in the crura of LP/J mice leading to conductive or combined hearing loss [5].

Histologically, three different phases can be distinguished: the first phase shows bone resorption. The tissue is highly vascularized and macrophages and activated osteoclasts are present. The second phase is characterized by new bone formation beginning around the vessels leading to the characteristic blue mantles of Manasse. Finally, in the last phase, the otosclerotic focus appears as a scar with rare cells and calcification [6].

Immunohistochemistry

A variety of immunocompetent cells including macrophages (MAC 387 antigen positive), HLA-DR-positive cells, cells expressing β_2-microglobulin, T suppressor cells and complement C3 were found in otosclerotic tissue by immunohistochemical investigations [7, 8]. Deposits of immunoglobulins (IgG, IgM and IgA) and complement C3 are present along the resorption lacunae, as well as in osteocytes and chondrocytes surrounding the destructive process [9].

What Is the Reason for This Inflammatory Process?

The etiopathogenetic hypothesis for the development of otosclerosis includes mechanical distress, enzymatic imbalance, disease of the collagen, and viral infection. The current hypothesis considers otosclerosis as an inflammatory disease with a genetic background. Five otosclerosis genes have been localized in familial cases of otosclerosis [10, 11], but the presence of these genes could not be confirmed by case-control studies. Mutations of collagen genes are also discussed as a cause for the otosclerotic process. However, a genetic inheritance is accepted in up to 50% of cases. The triggering event could be an environmental stimulus such as a common viral infection [12].

Electronmicroscopic studies in Paget's disease, which is histologically very similar to otosclerosis, revealed the presence of paramyxoviral structures in pagetic bone [13]. Analogously, filamentous structures very similar to paramyxoviral nucleocapsids were observed in otosclerotic bone specimen [14]. Immunohistochemical studies were undertaken to characterize these nucleocapsid-like structures. The expression of measles virus (MeV) N, F, and

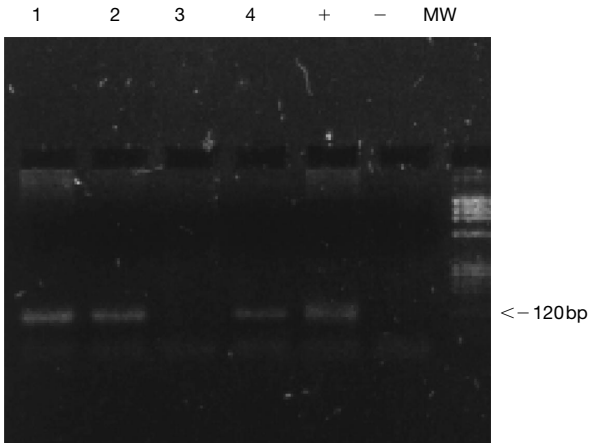

Fig. 1. Detection of MeV by RT-PCR. Lanes 1, 2, and 4 with the amplicons of the expected length (120 bp). MW = Molecular weight marker; + = positive control; − = negative control.

H antigens in osteoclasts and macrophages of otosclerotic tissue strongly supports the hypothesis that MeV is involved in otosclerosis [15–17].

Detection of MeV RNA within the Otosclerotic Tissue

Studies on the RNA level were undertaken to determine the presence of MeV-related sequences within the otosclerotic tissue since specificity and sensitivity of MeV antigen detection have been discussed controversially. Total RNA from fresh-frozen otosclerotic bone chips obtained during stapes surgery was analyzed by RT-PCR for MeV RNA (fig. 1) [18]. MeV-related sequences were amplified in an average of 84% in several studies, whereas the negative controls always remained negative [19–21]. A possible explanation for the negative cases is primarily the true absence of MeV in the otosclerotic tissue. Alternatively, the absence of an otosclerotic focus might explain the negative results, since RNA has been extracted from the stapes fragments without histologic controls. Finally, technical problems and limitations of the RNA extraction technique from small eburnized bone chips have to be considered.

McKenna et al. [22] were able to detect MeV RNA in 8 out of 11 temporal bones with morphologically confirmed otosclerotic foci. All negative controls remained negative. The true absence of MeV within the otosclerotic tissue might explain the 3 negative cases. However, false-negative results could be related to technical problems which may occur dealing with celloidin-embedded tissues.

Recently, Karosi et al. [23] have found MeV RNA in 14 out of 20 fresh-frozen footplates from patients with otosclerosis. They amplified RNA from minced and crushed bone chips by in vitro RT-PCR. In contrast, Grayeli et al. [24] could not confirm the presence of MeV neither in cells cultured from the otosclerotic foci nor in bone chips after RNA extraction and amplification by RT-PCR. They concluded that MeV is not involved in otosclerosis. However, it cannot be excluded that the negative results are due to the absence of otosclerotic foci in the examined tissue, as morphologic controls were not available. Furthermore, only few copies of MeV RNA are expected in a persisting infection and highly sensitive techniques including RNA extraction procedures are needed.

The controversial discussion about MeV RNA within the otosclerotic focus asked for a technique such as in situ RT-PCR, which combines morphology and amplification of the genetic material. In situ RT-PCR has been successfully used in research on hematologic tumors, but only few studies with bony tissue are available [25]. These studies were related to a paramyxoviral etiopathogenesis in Paget's disease and performed on decalcified bone. The authors demonstrated the presence of canine distemper virus in all cases examined [26, 27]. Up to now, we had analyzed stapes footplate specimens of 15 patients with clinical otosclerosis by in situ RT-PCR. The bone chips were decalcified and paraffin embedded and the histological examination demonstrated the presence of otosclerotic foci within the decalcified and paraffin-embedded tissue. In all cases, osteoblasts, osteoclasts, chondrocytes, and epithelial cells of the middle ear mucosa close to the otosclerotic focus contained MeV RNA amplification products [unpubl. data].

Recently, we have managed to genotype the MeV within the otosclerotic tissue. Cells cultured from otosclerotic bone chips of 5 patients had the morphological and biochemical characteristics of preosteoblasts. After RNA extraction and reverse transcription, the C-terminal part of the MeV N gene was amplified and sequenced by two independent companies. The phylogenetic analysis revealed that all MeV were of the genotype A. This genotype was present in Europe before the vaccination era and contains several wild-type strains isolated before 1970. Sequencing enabled us to distinguish MeV found in our patients from all other strains known up to now [unpubl. data]. This result proves the persistence of the MeV genome for more than 40 years within the temporal bone of patients with otosclerosis and excludes any speculation of contamination or false-positive results.

MeV Antibodies within the Perilymph

The otosclerotic focus usually has intimate contact with the perilymph spaces so that antigens from the otosclerotic focus might reach the immune target

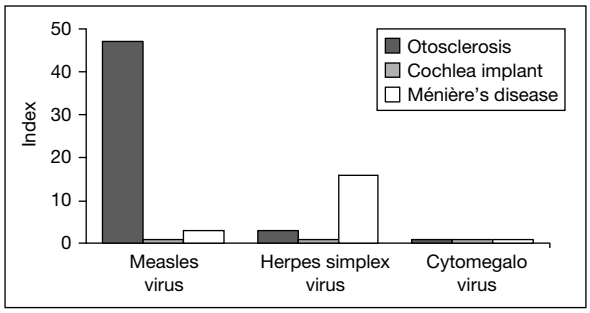

Fig. 2. Analysis of IgG in the serum and perilymph. Perilymph samples from patients with otosclerosis, or Ménière's disease, and from patients subjected to cochlea implantation were investigated by ELISA. The amounts of specific (MeV, herpes simplex virus, cytomegalovirus) IgG from total IgG in the perilymph in comparison with the amounts in the serum are expressed as index.

organ localized in the endolymphatic sac [28, 29]. It is known that antigenic stimulation of the endolymphatic sac via the perilymph can trigger a specific immune reaction [30]. We analyzed the perilymph and serum of patients with otosclerosis or Ménière's disease, and of controls for the content of albumin, total IgG and specific MeV IgG by nephelometric assay and ELISA. The MeV IgG fraction of total IgG was significantly higher in the perilymph compared to the serum of patients with otosclerosis (fig. 2) [21]. In contrast, evidence for local production of antibodies against herpes simplex virus type I was found in patients with Ménière's disease [31]. The reactivity of antibodies against MeV is decreased in patients with otosclerosis [32].

Conclusion

There is convincing evidence for a chronic inflammatory reaction in otosclerosis. MeV involvement was demonstrated in morphological, biochemical and immunological studies. Epidemiological data show a decrease in occurrence of otosclerosis and an increase in the average age of onset during the past 10 years, which could be due to the introduction of MeV vaccination in 1970 in Germany. Taken together, there is a strong association between MeV and otosclerosis. Further investigations will elucidate the role of MeV in the etiopathogenesis of otosclerosis.

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