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Etiopathogenesis of cholesteatoma

Received: 3 April 2003 / Accepted: 3 April 2003 / Published online: 27 June 2003

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Abstract Cholesteatoma is a destructive lesion of the temporal bone that gradually expands and causes complications by erosion of the adjacent bony structures. Bone resorption can result in destruction of the ossicular chain and otic capsule with consecutive hearing loss, vestibular dysfunction, facial paralysis and intracranial complications. Surgery is the only treatment of choice. The etiopathogenesis of cholesteatoma, however, is still controversial. This review was designed to understand the reasons for these disparities and to reduce or eliminate them. Future studies focused on developmental, epidemiological, hormonal and genetic factors as well as on treatment are likely to contribute to further understanding of cholesteatoma pathogenesis.

Keywords Cholesteatoma · Pathogenesis · Growth factors · Cytokines · Bone destruction

Introduction

Cholesteatoma, once established in the middle ear, mastoid or petrous bone, is a destructive lesion that gradually

expands and leads to complications by destruction of the adjacent structures. Erosion caused by bone resorption of the ossicular chain and otic capsule may result in hearing loss, vestibular dysfunction, facial paralysis and intracranial complications. Du Verney probably gave the first description of a cholesteatoma-like mass, which he called “steatoma,” in 1683 [37]. The French pathologist Cruveilhier defined cholesteatoma as “tumeur perleé,” or pearly tumor of the temporal bone, in 1829 [32]. The term “cholesteatoma” was coined by the German physiologist Johannes Müller in 1838, identifying a layered pearly tumor of fat [85]. The term “cholesteatoma” must be considered a misnomer as this benign tumorous lesion (“-oma”) contains neither cholesterine (“chol-”) nor fat (“-stea-”) [43, 124]. Though many otologists expressed dissatisfaction with this term, others such as “pearly tumor,” “margaritoma” or “keratoma” were not destined to last [134]. Three conditions seem to be necessary for the development of cholesteatoma: (1) the unique anatomical situation of the tympanic membrane, (2) chronic or recurrent infections and inflammation of the submucosal tissue in the middle ear and (3) mechanisms known from tissue repair.

Cholesteatoma is characterized by an intrusion of keratinizing stratified squamous epithelium into the middle ear. As soon as the epithelium begins to hyperkeratinize, the destructive behavior of cholesteatoma is triggered [141]. The discussion about how epidermic structures get into the middle ear is still controversial. The present report summarizes and evaluates theories and hypotheses on the etiopathogenesis of cholesteatoma.

Epidemiology

The annual incidence of cholesteatoma was found to be about 3 per 100,000 in children and 9.2 per 100,000 in adult Caucasian inhabitants in Finland and Denmark. There is male predominance [57, 104, 105, 148]. The incidence is about 1.4 times higher in males than in females (age groups: <50 years). The mean +/- SD age of children with congenital cholesteatoma is 5.6+/-2.8 years, while that of children

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with acquired cholesteatoma is 9.7 ± 3.3 years [88]. Epidemiology shows a high prevalence of cholesteatoma among Caucasians followed by people of African descent, while it is scarcely seen in (Non-Indian) Asians [104]. According to Kempainen et al., cholesteatomas show the same prevalence in all social groups [57]. Interestingly, it was confirmed that the prevalence of cholesteatoma in the Inuit Eskimos is significantly lower than average. The most likely explanation for this observation is that the larger nasopharynx in this ethnic group facilitates middle ear aeration and prevents the sequelae of chronic ear disease [107].

Classification

Since the initial stages of research in cholesteatoma there have been numerous discussions about its classification. Classically, three forms have been distinguished: (1) One "type" occurs behind an intact tympanic membrane and is often called "congenital," "primary" or "genuine" cholesteatoma. (2) "Primary acquired" cholesteatoma appears as a limited diverticulum of the pars flaccida, and there is little or no history of otorrhea. (3) "Secondary acquired" cholesteatoma appears with posterosuperior eardrum perforations and extension of the disease process into the antrum, mastoid, attic and middle ear. Granulation tissue, polyps and foul-smelling otorrhea are common [79, 158]. A subsequent classification, according to Tos, is based on the site of origination of cholesteatoma, which is an important factor for the surgical procedure and prognosis: (1) attic cholesteatoma, defined as a retraction of the pars flaccida or Shrapnell's membrane (Fig. 1) extending into the attic or aditus, and eventually into the antrum, mastoid or tympanic cavity, (2) sinus cholesteatoma, as a posterosuperior retraction or perforation of the pars tensa extending to the tympanic sinus, posterior tympanum and beyond and (3) tensa cholesteatoma, as a retraction and adhesion of the entire pars tensa involving the tympanic orifice of the Eu-



Fig. 1 Tympanic membrane showing a pars flaccida or Shrapnell's membrane cholesteatoma (48-year-old patient)

stachian tube (it may also extend further into the attic) [145]. In 1964, the Committee of Conservation of Hearing of the American Academy of Ophthalmology and Otolaryngology published a detailed classification for surgery of chronic ear infections [31]. In 1986, Meyerhoff and Truelson attempted to classify cholesteatoma according to its pathophysiology, location, eustachian tube, ossicular defects and presence or absence of complications. They divided the lesions into [77]: (1) primary acquired, (2) secondary acquired, (3) tertiary acquired and (4) congenital. In 1988, Tos proposed an otoscopic classification, which divided cholesteatoma into [144]: (1) attic, (2) pars tensa I (marginal disease) and (3) pars tensa II (central disease). Mills et al. added the fourth category to the Tos classification to include cholesteatomas occurring behind an intact tympanic membrane [82].

Lesions occurring within the temporal bone were classified by Sanna in 1993 into [119]: (1) supralabyrinthine, (2) infralabyrinthine, (3) massive labyrinthine, (4) infralabyrinthine-apical and (5) apical types. According to the sites affected with cholesteatoma, it is useful to classify: S1 for the site where cholesteatoma has started, S2 for when the disease spreads to another site, S3 for when the disease spreads to the third site, S4 for when the disease spreads to the fourth site and S5 for cases in which the primary site and four or more sites are involved. This classification distinguishes between seven sites: the attic and antrum, middle ear, mastoid, eustachian tube, labyrinth and middle fossa [118]. This staging is a practical solution for describing the extent of disease and is clinically relevant. It can be applied to all lesions except for those of the petrous apex, which cannot be diagnosed otoscopically [118].

With the advancement of reconstructive ossicular surgery, a classification of the condition of the ossicular chain is essential to compare different surgical procedures in relation to the primary disease process. The classification is a modification of that of Wullstein and Austin [11, 118, 159]: O0 if the ossicular chain is intact, O1 if the incus is eroded with discontinuity of the ossicular chain, O2 if the incus and stapes arch are eroded and O3 if the malleus handle and incus are absent and the stapedial arch is eroded.

Another classification considers preoperative complications: It is usually related to the stage of disease and is important during management. Five complications are observed: fistula of the lateral semi-circular canal, facial palsy, total sensorineural hearing loss, sinus thrombosis and intracranial invasion. Three stages are distinguished [118]: C0 for no complications, C1 for occurrence of one complication and C2 for two or more complications.

Tos also suggested a classification of acquired cholesteatoma based on the site of appearance of cholesteatoma: attic cholesteatoma, sinus cholesteatoma and tensa cholesteatoma [147]. Combining otoscopic evaluation of the extent of the lesion, assessment of ossicular condition and the preoperative complications is useful to determine the surgical procedure and compare the results of surgery.

A profound understanding of the pathogenesis of middle ear cholesteatoma is particularly important, since it is the destructive nature of this lesion that is responsible for

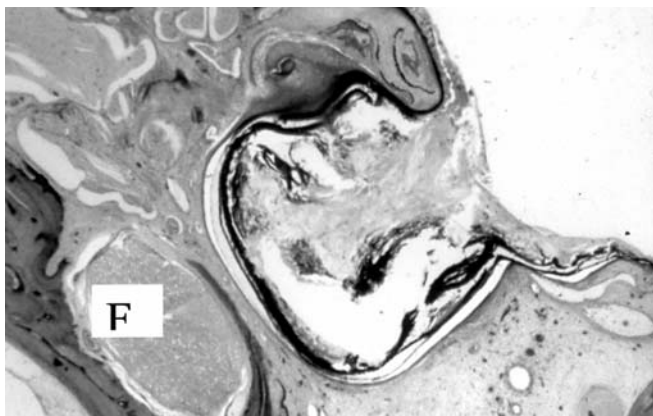


Fig. 2 Cholesteatoma adjacent to the facial nerve (*F*) (with permission from Lange's temporal bone collection, Leipzig, Germany, HE, $\times 20$)

most of its complications (Fig. 2). The tendency of cholesteatoma to erode bone and the lack of effective, nonsurgical treatment underline the importance of investigating the basic pathomechanisms of this disease. It is generally accepted that cholesteatomas may be classified according to their theoretical mechanism of pathogenesis into two general categories: congenital or acquired.

Congenital cholesteatoma: definition and theories on pathogenesis

Congenital cholesteatomas have been defined by Derlacki and Clemis as embryonic remnants of epithelial tissue behind an intact tympanic membrane, the patients having no prior history of ear infection or surgery [35] (Fig. 3). Although this definition is widely accepted, it is associated with several uncertainties, as it is essentially a diagnosis of exclusion. Intratympanic cholesteatomas are more common in children than in adults, as is generally expected in congenital diseases [35]. Until the publication of a number of relatively large case series during the last decade [69, 97, 103], congenital cholesteatoma was generally considered to be extremely rare. Congenital cholesteatoma generally grows in the anterior superior quadrant, initially into the posterior superior quadrant and the attic and finally into the mastoid [63]. Spreading within three distinct anatomical sites suggests a natural classification into three types [88]: type 1 if lesions are limited to the middle ear but do not involve the ossicles, except for the malleus manubrium; type 2 if lesions involve the ossicular mass in the posterior superior quadrant and the attic; type 3 if lesions involve the mastoid. Type 1 is controlled by extended exploratory tympanotomy and does not require a second-look reexploration. Type 2 lesions are approached by an extended tympanotomy, but may necessitate atticotomy and canal wall-up tympanomastoidectomy (with or without opening of the facial recess). They require a second-look reexploration. Ossicular reconstruction may be necessary. Type 3 lesions are approached in the same manner as type 2 lesions, but

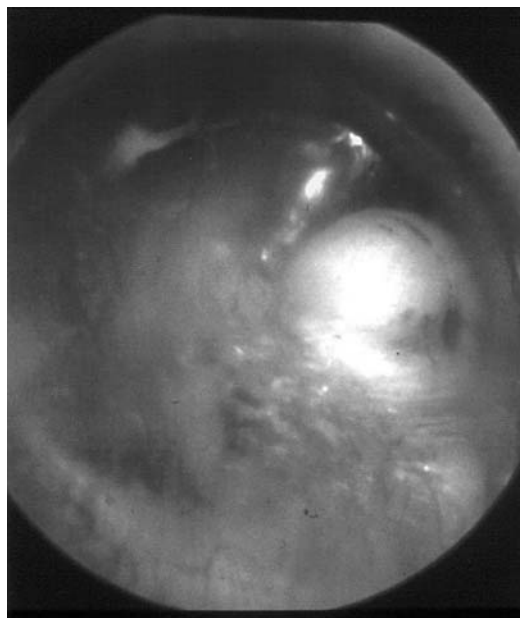


Fig. 3 Congenital cholesteatoma behind an intact tympanic membrane in a 3-year-old boy

occasionally a canal wall-down tympanomastoidectomy could be necessary. As the type progresses from 1 to 3, the frequency of recurrence increases [88]. Two histologic types of congenital cholesteatoma, an open and closed form, have been described by Karmody et al. [54].

Theories on etiopathogenesis of congenital cholesteatoma

Lucae observed cholesteatoma that appeared to originate from the mucosa of the tympanic cavity in a patient with a normal middle ear and tympanic membrane [72]. This seems to be the first description of what is now considered to be a congenital cholesteatoma. Many theories have been published about the origin of congenital cholesteatoma. In 1854, von Remak postulated that the origin of dermoids and other related tumors is a follicle of skin that has been dislocated during early embryonic life [108]. In 1855, Virchow suggested that congenital cholesteatoma originates from connective tissue [152]. Ruedi postulated that inflammatory injury to the tympanic membrane of the fetus produces microscopic perforations through which the proliferating basal epidermal cell layer invaginates into the middle ear to form a congenital cholesteatoma [113]. Papparella and Rybak suggested that ectodermal implants result from fusion planes of the first and second branchial arches [96]. Aimi proposed an epithelial migration theory assuming that ectodermal cells of the developing external auditory canal accidentally migrate through the tympanic isthmus into the middle ear where they can form congenital cholesteatoma [4]. Another theory was presented by Northrop et al., who hypothesized that viable epithelial cells found in the amniotic fluid could be the source of



Fig. 4 “Epidermoid formation” (*arrow*) in a fetal temporal bone of the 33rd gestational week (HE, $\times 100$) (with permission from Professor L. Michaels, London)

congenital cholesteatoma [90]. Teed in 1936 and Michaels in 1986 identified an epidermoid remnant in the anterosuperior quadrant of the middle ear of temporal bones of human fetuses, which Michaels called the epidermoid formation (EF) [78, 143] (Fig. 4). It could not be found after the 33rd week of gestation, and it was postulated that its persistence results in the development of congenital cholesteatoma. A recently published paper by Karmody et al. gives histological evidence of the squamous epithelial remnant in two postpartum patients [54]. McGill et al. and Wang et al. confirmed the presence of the epithelial remnant in fetal temporal bones, but could not find evidence of keratinization [75, 153]. Keratinization was never observed in any EF by Kayhan et al. [56]. Contrary to previous reports, they found EFs not only in ears of fetuses, but also in ears of infants and children. Although EFs may persist in some ears, possibly developing into congenital cholesteatomas, our findings do not provide direct support for this concept.

A new acquired inclusion theory presented by Tos is based on the fact that the place of origin of the anterosuperior mesotympanic cholesteatoma is the area of the malleus handle and malleus neck, and of the posterosuperior cholesteatoma, the long process of the incus. Under common pathological conditions, the risk of retraction and adhesion of the eardrum to these ossicles is high. Some of the keratinized squamous epithelial cells may be left behind, trapped in the tympanic cavity forming an inclusion cholesteatoma [146]. Levine et al. recently published an-

other case of postnatal persistence of epidermoid remnants in the human middle ear [70]. However, a clear transition from epidermoid formation to unequivocal cholesteatoma has not yet been demonstrated.

Lee and co-workers found evidence that epidermoid formations are often located close to a developmental epithelial fold, which could close off to produce a cyst [68]. These features support the concept that the epidermoid formation is a precursor of the small anterosuperior congenital cholesteatoma [80].

The reason for persistence of the epidermoid formation in some cases is, of course, subject to speculation. A certain event in cellular regulation that normally results in the regression of the epidermoid formation, or its transformation into middle ear epithelium, may fail to occur. Thus, the unambiguous demonstration of the transition of an epidermoid formation into cholesteatoma must remain subject to further studies.

Primary epidermoid cysts are congenital cholesteatomas [106]. These epithelial remnants of the embryonic period may occur throughout the body with the highest prevalence in the meninges, the brain, the skull base and the temporal bone. This lesion resembles acquired varieties morphologically in that it shows concentric layers of keratin in a cavity that is lined by squamous epithelium and encapsulated by pseudoconnective stroma. Primary epidermoid cyst occurring in the temporal bone, in other bones of the skull base, within the meninges, the brain and in other parts of the body is known as a congenital cholesteatoma [106]. The pathology is similar to that of the acquired varieties: a squamous epithelial-lined cavity filled with concentric layers of keratin. A pseudoconnective stroma encapsulates it. Progressive exfoliation of keratinous material into the interior of the cyst produces a waxy white material that leads to a slow expansion of the cyst [106]. Viable squamous epithelial cells originating from the amniotic fluid have been found present in the middle ears of neonates. However, the authors found no evidence that these cells could implant into the middle ear to form a congenital cholesteatoma [90].

The epithelial migration theory suggests that ectoderm from the embryonic external auditory canal surmounts the hypothetical restrictive mechanism of the tympanic ring and migrates into the middle ear [4]. This theory could be a possible explanation for the occurrence of congenital cholesteatoma in the posterior and inferior parts of the middle ear, close to the site of the embryonic tympanic ring [63]. Kountakis et al. found that epidermoid cells from the cerebellopontine angle *in vitro* exhibit migratory properties similar to those of cholesteatoma cells from the middle ear and mastoid cavity. This includes mass migration, a variable migratory rate, random direction of migration and a similar migratory profile. These migratory properties are unique to epithelium derived from the first brachial groove system such as epithelium of the tympanic membrane and cholesteatoma of the middle ear and mastoid [64]. Congenital cholesteatoma could derive from nests of squamous epithelial cells, which occasionally keratinize in the lateral wall of the embryonic tympanic cavity below the

level of the pars flaccida. Such epidermoid formation is usually present between the 10th and the 33rd gestational week [78]. The cause of a congenital cholesteatoma could also be remnants of occasionally keratinizing squamous epithelial cells in the lateral wall of the embryonic tympanic cavity below the level of the pars flaccida. These remnants of cells, called epidermoid formations, were observed as early as the 10th week of gestation and consistently disappeared until the 33rd week of gestation [78]. However, discussion about whether the majority of cholesteatomas in the anterior mesotympanum are congenital or acquired remains controversial [63, 146].

The origin of congenital cholesteatoma remains uncertain, but a substantial body of evidence indicates that most congenital cholesteatomas originate as embryonic epidermoid formations in the anterior mesotympanum that fail to involute [63]. Tos presented several clinical and histological reasons for doubts about the epithelial formation theory, rather supporting the acquired inclusion theory as an explanation of the mesotympanic cholesteatoma [146].

Recently, attention has been focused on the telomere length to establish a molecular biological approach for differentiating between congenital and acquired cholesteatoma. Telomeres in congenital cholesteatomas are shorter than those in the acquired variant, which shares telomere length with inconspicuous skin cells of the external ear canal. The level of telomerase activity in congenital cholesteatoma is low. The telomere length in congenital cholesteatoma is shorter than in normal external ear canal skin, whereas in acquired cholesteatoma tissue, the telomere length is almost the same as in normal external ear canal skin. Congenital cholesteatoma may originate from fetal-stage tissue remnants or aberrant tissues [62].

Clinical features of congenital cholesteatoma

Although acquired and congenital cholesteatoma are likely to derive from different pathomechanisms, there is evidence that some cases of congenital cholesteatoma, if untreated, grow and become indistinguishable from acquired cholesteatoma. Congenital cholesteatomas may expand and become super-infected, resulting in bone destruction and chronic ear infection similar to that caused by acquired cholesteatoma [45].

Congenital cholesteatoma in industrialized countries is associated with a lower incidence of intracranial complications, a relatively higher incidence of facial nerve impairment and ossicular destruction and wide-spread malformations, but better postoperative hearing results than the acquired form [34].

Acquired cholesteatoma: definition and theories on pathogenesis

Pathogenesis of “acquired” or “secondary” cholesteatoma has been subject to extensive debates for more than a century. There is evidence for at least four different mecha-

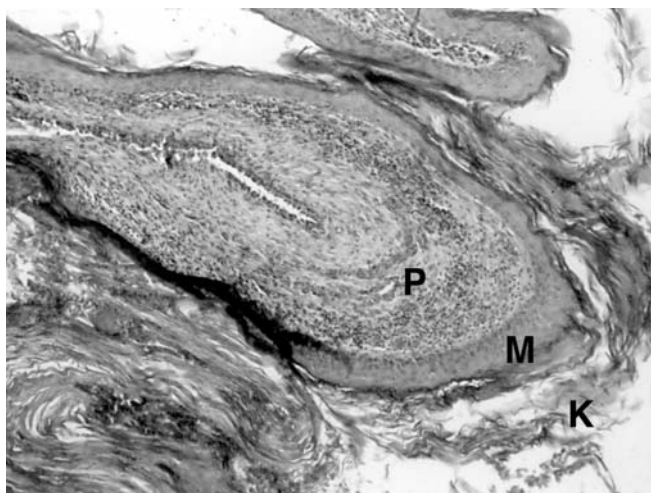


Fig. 5 Histology of acquired cholesteatoma of middle ear. It is composed of a keratinous layer (*k*) developed from an outer epidermoid layer (*matrix*) (*m*) and the inflamed subepithelial connective tissue (*perimatrix*) (*p*) (HE, $\times 200$)

nisms for the development of this disease: (1) squamous metaplasia of middle ear epithelium due to infection, (2) epithelial invasion/immigration through a perforation of the tympanic membrane, (3) invasive hyperplasia of the basal cell layers, (4) retraction pockets and invaginations of the tympanic membrane due to eustachian tube dysfunction and (v) a conceivable combination of the mentioned theories [67, 145] (Fig. 5).

Squamous metaplasia theory

In 1864, von Trötsch regarded the stratified keratinizing squamous epithelium of cholesteatoma as a metaplastic product of the middle ear mucosa under the pressure of dried, caseous pus [149]. Wendt (1873) suggested that the non-keratinizing epithelium of the middle ear and the mastoid could undergo a metaplastic transformation into keratinizing epithelium. This could result in cholesteatoma as a result of inflammation [154]. Sadé supported this theory and pointed out that the pluripotent middle ear epithelium could be stimulated by inflammation into a metaplastic stratified squamous epithelium with keratinization [115]. The metaplastic epithelium grows because of the accumulation of keratin, and recurrent infection and inflammation leads to lysis and perforation of the tympanic membrane resulting in the typical appearance of attic cholesteatoma. The metaplasia theory is supported by the observation that middle ear mucosa biopsies obtained from pediatric otitis media patients with effusion occasionally display islands of keratinizing epithelium [114]. Chole and Frush described metaplasia of the normal middle ear mucosa into a lightly keratinizing squamous epithelium in rats under extreme vitamin A deprivation [25]. However, there is no direct histological or experimental evidence that these mechanisms lead to cholesteatoma formation [42].

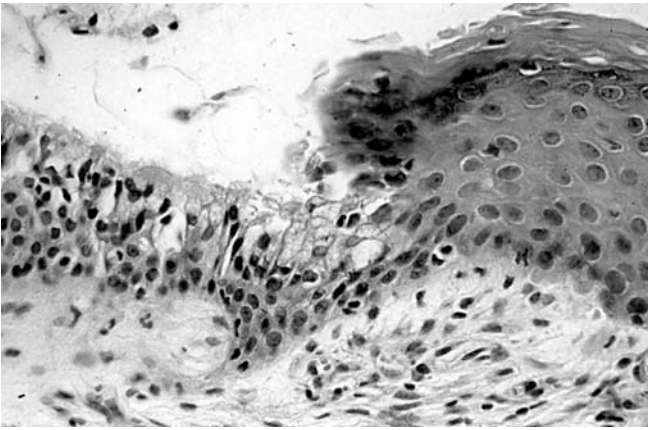


Fig. 6 Mucocutaneous junction between cholesteatoma, epithelium and middle ear mucosa. (HE, $\times 400$)

Immigration theory

Habermann in 1888 and Bezold in 1890 proposed the epithelial immigration or invasion theory, suggesting that squamous epithelium migrates from the margin of a tympanic membrane perforation into the middle ear spaces [15, 46]. In 1901, Politzer observed that the epidermis of the external auditory canal can grow over a perforation of the tympanic membrane into the middle ear cavity. He assumed that after perforation of the tympanic membrane, a second infection would be the cause of epithelial migration [46], (Fig. 6). Immigration of epithelium from the tympanic membrane into the middle ear of infected guinea pig bulla resulting in cholesteatoma was shown by Friedmann [42]. Similarly, Steinbach, Wright et al. and Masaki et al. demonstrated that a mixture of propylene glycol and antibiotics caused perforations and immigration of epidermis into the middle ear of chinchillas and rabbits [73, 130, 156].

Basal hyperplasia theory

Lange observed in 1925 that epithelial cells from the keratinizing epithelium of the pars flaccida could invade into the subepithelial space and form an attic cholesteatoma [67]. Ruedi provided experimental and clinical evidence for this theory of cholesteatoma pathogenesis [113]. Further studies with human specimens by Lim et al. and also animal experiments have confirmed that the basement membrane focally dissolves and gives access for the keratinocytes to invade the subepithelial space [26, 42, 71, 157]. Pseudopodia from the basal cell layer seem to break the basal lamina, allowing epidermal keratinocytes to invade the lamina propria [137]. The entrapped cells keratinize and form inclusion cysts that may expand and form cholesteatoma.

Retraction pocket or invagination theory

The retraction pocket or invagination theory was presented by Wittmaack in 1933 and is generally accepted as the most common pathogenetic mechanism in acquired cholesteatoma [155]. According to this theory, the pars flaccida, or occasionally the pars tensa, of the tympanic membrane retracts into the middle ear. The underlying pathomechanism of retraction pocket formation is likely to be related to negative pressure, inflammation or both. The retention of accumulating keratin within the deep retraction pocket establishes cholesteatoma. The loss of drainage of the retraction pocket is thought to induce the expansion into the middle ear and mastoid spaces. An altered epithelial migration pattern in these deep retraction pockets was observed by Sadé [115, 116].

Clinically, each of these pathogenetic mechanisms appears to account for acquired cholesteatoma. Although there is experimental and observational evidence for each of the four mentioned theories, none of them is unequivocally proven.

The pathogenesis of two further types of acquired cholesteatoma is quite apparent: (1) the iatrogenic cholesteatoma, manifesting after previous surgery for non-cholesteatomous middle ear disease, like tympanoplasty or grommet insertion and (2) posttraumatic cholesteatoma, which originates from trauma scattering squamous epithelium into the middle ear [76, 160].

Topology and subclassification of acquired cholesteatoma

Attic cholesteatoma

Attic cholesteatoma is defined as a retraction of the pars flaccida or Shrapnell's membrane extending into the attic and eventually into the antrum, mastoid or tympanic cavity [147]. Several theories on the pathogenesis of attic cholesteatoma have been framed: the retraction theory, the papillary proliferation theory, the immigration theory and the metaplasia theory. A combination of the retraction theory and the proliferation theory has been suggested as an explanation for the pathogenesis of attic cholesteatoma [132]. The authors divided the pathogenetic process into several stages. The first stage is a retraction pocket. Most retractions will never progress to cholesteatoma because of migration of the squamous epithelium from the basal cell layer to the surface; thus, self-cleansing of the retraction pocket functions well. In a few retraction pockets, the keratinized epithelium starts to proliferate, and the self-cleansing mechanism is disturbed. This may lead to accumulation of debris within the retraction, crust formation and local inflammation behind the crust. Clinically, this condition could be regarded as precholesteatoma. At this point, the proliferation stage of the retraction pocket begins. It leads to cone formation: The basal keratinocytes proliferate resulting in elongation of epithelial cones. Once a cone is established, the intraepithelial maturation of the keratinocytes is differ-

ent from that of normal skin. Keratin accumulates in the center of the cone. Under the pressure of the keratin, the microcholesteatoma increases in size and fuses, and the cholesteatoma expands. Expansion of several microcholesteatomas is the next stage in the pathogenesis of attic cholesteatoma. The bottom of the retraction pocket is open and the cholesteatoma grows deeper into the middle ear spaces in this stage. Once the expansion stage of attic cholesteatoma is established, bone resorption of the adjacent ossicles and scutum is initiated [132].

Sinus cholesteatoma

Sinus cholesteatoma is defined as a postero-superior retraction or perforation of the pars tensa extending to the tympanic sinus, posterior tympanum and beyond [147].

The sinus tympani has been the focus of clinical interest because cholesteatoma has the tendency to invade it. It was found that the sinus tympani is bounded laterally by a constant ledge of bone anterior to the facial nerve. The microscope enables the surgeon to examine this region because the orifice of the retraction pocket is more or less perpendicular to the axis of the external auditory canal. Authors emphasize the importance of removal of the lateral lip of bone to expose the orifice of the sinus tympani [12].

Tensa cholesteatoma

Tensa cholesteatoma is defined as retraction and adhesion of the entire pars tensa involving the tympanic orifice of the eustachian tube. It may also extend further into the attic [147]. Lesions involving the pars tensa are not uncommon in clinical practice [128]. Mechanisms such as metaplasia, ectopic epidermis remnants or ingrowth of meatal epidermis have been proposed to explain the pathogenesis of cholesteatoma behind an intact tympanic membrane. Histopathologic findings in temporal bones emphasize the role of an acquired epidermal remnant. Cholesteatoma behind an intact tympanic membrane can originate from a resolved retraction of the pars tensa [133]. Retraction of the pars tensa minimizes middle ear volume and thus according to Boyle's law (volume x pressure is constant) acts as a pressure buffer. A direct correlation was found between the degree of atelectasis and the middle ear volume replaced by the atelectasis. Retraction of the pars tensa may counteract negative middle ear pressure, depending on the degree of retraction and the extent of mastoid pneumatization [117]. The management includes surgical procedures ranging from grommet insertion to extensive tympanoplasty [128].

Methodological concepts in cholesteatoma research

Regardless of their underlying pathogenesis, all cholesteatomas share similar properties. Their common clinical

hallmarks are invasion, migration, hyperproliferation, altered differentiation, aggressiveness and recidivism. Animal experiments, clinical trials, cell and tissue cultures as well as the application of modern technologies (e.g., immunohistochemistry, in-situ hybridization, polymerase chain reaction and microarrays) might improve understanding of the biological properties and the pathophysiology of this lesion.

Epithelial proliferation and differentiation

Pathogenesis of acquired and congenital cholesteatoma brings up two fundamental questions: (1) what is the origin of the keratinizing squamous epithelium and (2) what influences invasive and hyperproliferative behavior of the epidermis?

Cholesteatoma can be regarded as a disorder of growth control. This allows the formulation of two different hypotheses. It is generally accepted by now that the stratified squamous epithelium is derived from the epidermis of the tympanic membrane and external canal skin. With regard to the second question, the behavior of cholesteatoma tissue may be induced by a loss of growth-inhibiting signals, by an increase in growth-promoting factors or both of these changes. Before conclusions like these can be made, it is necessary to objectively confirm the well-known clinical observation that cholesteatoma shows high growth activity.

Cell cycle analysis and proliferation markers

Several comparative investigations have been performed to assess the epithelial cell kinetics of cholesteatoma using normal meatal skin as a control (Fig. 7). In one of these studies, MIB 1 (Ki-67) immunostaining, which reacts with a nuclear antigen expressed by dividing cells, was applied [136]. Specimens of normal auditory meatal skin expressed an average MIB 1 score of 7%. Cholesteatoma samples expressed an average MIB 1 score of 17% and heterogeneity of proliferating epithelial areas [136] (Fig. 7). Epithelial cones growing toward the underlying stroma exhibited high mitotic activity. The results of this study confirm a highly significant increase in the proliferation rate of cholesteatoma keratinocytes with an MIB 1 score that was 2.3 times higher than the score for keratinocytes of normal external auditory meatal skin.

As a result of the various approaches to evaluating proliferative activity, cytokeratin 13/16, proliferating cell nuclear antigen (PCNA) and thrombomodulin (TM) have recently been introduced as markers of cellular proliferation [100]. PCNA is a 36 kD nuclear antigen that appears at S and G1 phases of the cell cycle [16]. PCNA positive cells have been detected in the suprabasal and parabasal layer as well as in the basal layer of cholesteatoma epithelium. Compared to normal skin, cholesteatoma epithelium exhibits a significantly higher percentage of PCNA positive cells. Staining intensity was different in the various areas

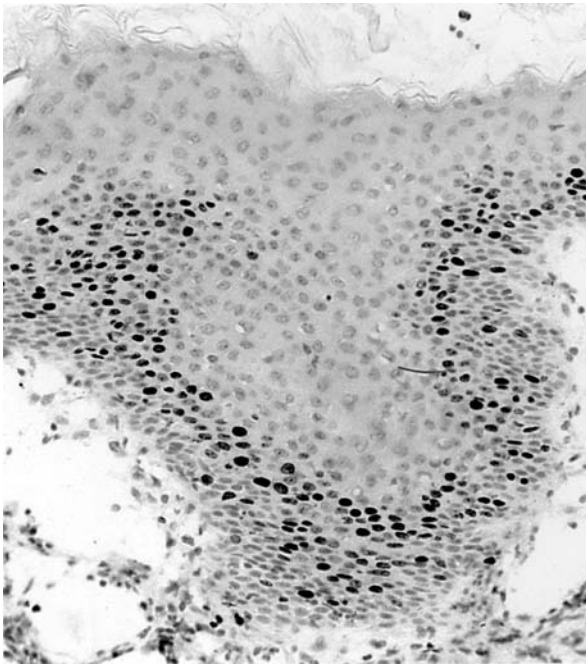


Fig. 7 MIB1-positive, proliferating keratinocytes of cholesteatoma matrix. Growth cone infiltrating the subepithelial connective tissue (HE, $\times 200$)

of the epithelium and did not depend on the thickness of the epithelium [91]. Park et al. conducted studies on induced cholesteatoma in Mongolian Gerbils demonstrating that induced cholesteatoma keratinocytes have higher mitotic capacity than normal keratinocytes also seen in the suprabasal layer. These studies suggest that extensive accumulation of keratinous mass in the external auditory meatus may induce changes in cellular differentiation and proliferation. The authors explain the increased mitotic activity by the high number of newly formed keratinocytes in the suprabasal layer as well as in the basal layer and by the increased turnover rate of the basal cells [99]. As the experiments conducted by Soyer et al. in specimens of psoriasis and melanocytic skin tumors demonstrated, antigen Ki-67 is a marker of cell proliferation of the epidermis [127]. The presence of Ki-67 positive cells has been observed in the suprabasal layer and stroma of cholesteatoma in a significantly higher percentage than in normal skin [20, 91]. Thrombomodulin (TM) is a cell surface glycoprotein that forms a high affinity non-covalent complex with thrombin and is a differentiation marker for spinous layer keratinocytes [84]. Therefore, strongly positive staining for TM in the suprabasal layer is very meaningful [100]. Quantification of the proliferating activity of the epithelium allows the estimation of cholesteatoma aggressiveness, the intensity of bone destruction and the likelihood of recurrence [22]. Further studies using proliferation markers such as PCNA, AgNOR (argyrophilic nuclear organizer regions) and thymidine labeling showed comparable results and proved the high proliferative activity of cholesteatoma epithelium [22, 93, 136, 139]. For instance, Sudhoff et al. observed a similar proliferative activity in both

the congenital and the acquired variant of cholesteatoma in AgNOR staining [139].

Having in mind the observation that cholesteatoma does have such a high proliferative activity, it is necessary to inquire if it can be a form of low-grade neoplasia. Analysis of the DNA content of cholesteatoma could provide evidence for or against this hypothesis.

DNA content

In a study performed by Desloge et al., the DNA content of ten cholesteatoma and six postauricular skin specimens was compared using flow cytometry; the DNA content of some cholesteatoma specimens was also analyzed using image analysis [36]. In one cholesteatoma specimen, an abnormal aneuploid DNA content was found, whereas the remaining nine cholesteatomas and the six postauricular skin specimens had a normal euploid DNA content. The authors conclude that considering the lack of overt genetic instability as evidenced by the presence of a normal euploid DNA content, cholesteatomas cannot be regarded as low-grade neoplasms [36].

Lipids

Cholesteatomas do not contain fat, although biochemical examinations of the matrix have revealed high concentrations of cholesterol and cholesterol ester [47, 112]. Cholesterol imprints may be seen histologically. The intracellular lipids are present in the keratinocytes of the stratum granulosum. They are synthesized and stored in the Odland bodies. Extracellular lipids in cholesteatoma are metabolized from the contents of Odland bodies and consist of fatty acids, ceramides and cholesterol. The synthesis of barrier lipid is normally controlled by the composition of the barrier. Immediately after barrier breakthrough, signal molecules are liberated that start the cytokine cascade. The balance between lipid secretion and lipid loss is disturbed in cholesteatomas [38, 89]. Defective growth signal transduction and increased cytokine expression has been demonstrated in cholesteatoma [86, 142].

Cytokeratins and differentiation markers

Several studies have shown the invasive and hyperproliferative behavior of epidermis in cholesteatoma. It could be demonstrated to be surely benign. As a next step, it is necessary to ask for the origin of the keratinizing squamous epithelium in cholesteatoma. To investigate this question, numerous immunohistologic studies have been performed to compare the localization of markers of epidermal differentiation in cholesteatoma and normal external ear canal skin [19, 65, 151]. Cytokeratins (CK) are intermediate filament proteins that are exclusively present in epithelial cells. The expression of different types of CKs (numbered 1–20) varies with the type of epithelium and the

stage of differentiation. Because of these properties, CKs seem suitable for a better understanding of the mechanisms of squamous cell metaplasia of the middle ear.

As the pathogenesis of cholesteatoma (migration versus metaplasia) has been subject to vigorous debates, similar cytokeratin (CK) patterns could be demonstrated in keratinocytes of cholesteatoma, normal auditory canal skin and tympanic membrane skin [65]. Kakoi and co-workers studied the differentiation markers involucrin and filaggrin as well as glycoproteins and found an abnormal distribution of all three of them in both basal cells and suprabasal cell layers of cholesteatoma [53].

However, not all of the cholesteatomas showed hyperproliferative properties: The expression of CKs 6, 16 and 19 in cholesteatoma epithelium showed hyperproliferative patterns. Patterns of the terminal differentiation of CKs 1, 5, 10 and 14 in cholesteatoma were basically the same as those in skin. In cholesteatoma, each CK gradually decreased in molecular weight in the granulated layer and debris [53].

Using gel electrophoresis and immunohistochemistry, Bujia and co-workers demonstrated the presence of cytokeratin 16 in cholesteatoma samples [19]. Their results provided additional support for the hyperproliferative character of cholesteatoma epithelium.

Analyzing the cytokeratin pattern, Kujipers et al. concluded that the matrix of cholesteatoma is not the result of a metaplastic change. They found cholesteatoma epithelium similar to that of tympanic membrane and ear canal skin, but in various states of proliferation, dependent on the degree of inflammation present [65]. The cytokeratin patterns are known to be affected during the formation of aural cholesteatoma. In a recent study in an animal model of aural cholesteatoma, Kim and Chen employed immunohistochemical methods to investigate the distribution of cytokeratin and the binding patterns of lectin [58]. They conclude that the origin of aural cholesteatoma may be external auditory canal epidermal cells and that the characteristics of these cells do not change during cholesteatoma development. According to Kim et al., the patterns of cytokeratin expression correspond well with the state of keratinocyte proliferation, migration and differentiation. The authors used an animal model with experimental induction of cholesteatomas in gerbils by external auditory canal ligation (ECL). This animal model allowed the investigation of CK expression in various clinical stages of cholesteatoma. The results of this study support the concept that the normal keratinizing epithelium of the tympanic membrane undergoes certain changes as it forms a cholesteatoma in the external canal and middle ear. An increased expression of CK5/6 and CK13/16 is found in the suprabasal epithelial layer of the external auditory canal in stages I through III cholesteatoma. This gives further evidence that cholesteatoma epithelium shows increased proliferation of keratinocytes, especially in the pars tensa of the tympanic membrane near the malleus. Additionally, the increased expression of CK4 in the external meatal skin close to the tympanic membrane and the transition of the CK13/16 expression from the basal to the suprabasal

epithelial layer suggest that the epithelium migration might be increased from the outer to the inner portion of the auditory canal [60].

Investigations by Kim and co-workers based on an animal model of induced retraction pocket cholesteatomas also revealed that aural cholesteatoma is a disease with a spectrum of pathological conditions. In this study, the authors found that CK expression is altered only in focal parts of retraction pocket cholesteatoma and that the significant CK change is located at sites adjacent to cholesteatoma, not in the cholesteatoma itself. This might explain that transmigration and hyperproliferation of squamous epithelium occurs in areas adjacent to the cholesteatoma [59].

Arriaga et al. used monoclonal antibodies against cytokeratin subtypes in cholesteatoma to try to inhibit growth of keratinocytes in case of residual disease [10]. They investigated the keratinocyte cytotoxicity of monoclonal antibodies directed against a cytokeratin subtype relatively unique to cholesteatoma. Their experiments showed that monoclonal antibodies do not have an *in vitro* activity against keratinocytes. However, additional investigation of a possible role of cytokeratin monoclonal antibodies is required with the goal of developing a clinically useful biologic adjunct for cholesteatoma management.

Tumor-suppressor genes, transcription factors and apoptosis

Cases have been described where congenital cholesteatoma underwent spontaneous elimination [62]. Not every ectodermal tissue invaded or remaining as a remnant in the mesenchymal tissue grows and forms cholesteatoma. Under normal conditions, such ectodermal tissue is naturally eliminated by means of apoptosis [62]. It has been proposed that the development of human cholesteatoma is due to the altered control of cellular proliferation, which tilts the balance toward the aggressive, invasive growth of squamous epithelium into middle ear. However, whether the cause of this altered control is a defect in the mechanisms and underlying genes that control proliferation, or cytokines released from infiltrating inflammatory cells or another mechanism, is unknown [8] (Figs. 8 and 9).

The nuclear phosphoprotein p53 tumor suppressor gene plays a pivotal regulatory role in cell cycle control and apoptosis. Congenital, primary, secondary acquired and recurrent cholesteatomas have been analyzed by Albino and co-workers for the altered expression of p53 [6]. Albino's experiments show that the expression of p53 in cholesteatoma is 9–20 times higher than in normal postauricular skin or tympanic membrane. A possible explanation for these findings could be a defect in the mechanisms that p53 engages (i.e., cell cycle control or apoptosis or both) in cholesteatoma. Furthermore, Albino's results suggest that there is no intrinsic difference between any clinical group of cholesteatomas, at least with respect to p53 expression and, presumably, p53 function [6].

Recent publications have shown that the c-jun protein promotes keratinocyte proliferation and p53 induces apop-

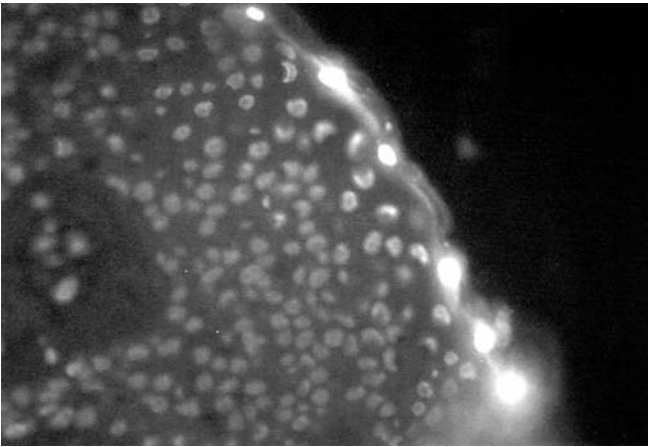


Fig. 8 TUNEL staining in normal retroauricular skin. A few apoptotic cells in the suprabasal layer of epidermis, $\times 400$

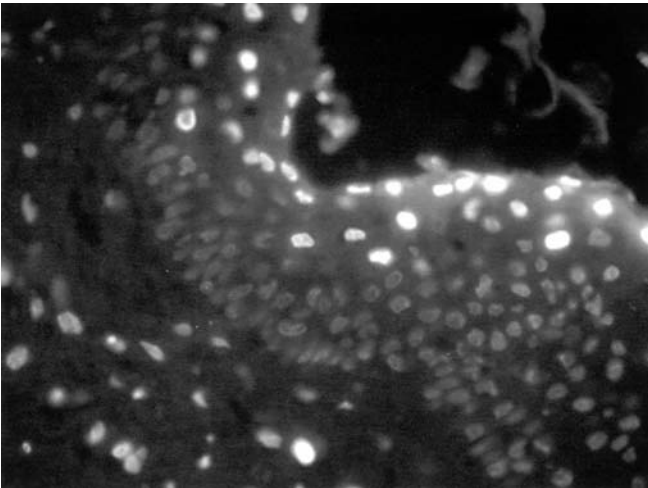


Fig. 9 Cholesteatoma. Numerous apoptotic cells seen in the suprabasal layer of cholesteatoma epithelium, $\times 400$

tos of various cells. C-jun functions as a transcription factor for many genes and the p53 protein functions as a negative regulator of cellular proliferation and is involved in the apoptosis pathway that induces DNA damage. In a study by Shinoda and co-workers, the presence of c-jun and p53 in cholesteatoma was demonstrated by immunoblotting assays [126]. Cholesteatoma tissue incubated with anti-c-jun antibody showed a staining of keratinocytes of the basal and spinous layers of the epithelium. The c-jun protein was located in the basal layer of normal skin, and the p53 protein was present in the nucleus of keratinocytes in the granular layer of cholesteatoma epithelium. Keratinocytes of normal external ear canal skin and normal human skin were only slightly stained in the granular layer of the epidermis. These results suggest that c-jun and p53 proteins may play a role in keratinocyte differentiation, proliferation and apoptosis in cholesteatoma [129].

An increased rate of keratinocyte cell death compared to normal skin causes an accumulation of keratin debris in the middle ear. The death of keratinocytes (terminal differ-

entiation) in the epidermis may constitute a form of apoptosis [102]. In recent studies, apoptotic cells were found in the suprabasal layers of cholesteatoma epithelium in a higher percentage than in normal skin [91, 98]. The preliminary data of a study conducted by Choufani and co-workers indicate that cholesteatomas with a high apoptotic index are the most prone to recurrence. The distribution of apoptotic cells in the cholesteatomas' epidermal compartment was the second most reliable variable in the prediction of recurrence of the disease selected by discriminant analysis. The distribution of apoptotic cells in non-recurrent cholesteatomas was more heterogeneous than in recurrent ones. This also means that recurrent cholesteatomas exhibit a higher fraction of apoptotic cells [28].

According to Miyazaki et al., the process of terminal differentiation in the cholesteatoma epidermis proceeds in the same manner as in normal skin. Moreover, according to this study, the following scenario of events appears conceivable: First, while the cholesteatoma epidermis is in a state of hyperproliferation due to the elevation of the levels of various cytokines, regulation is achieved through normal progression of terminal differentiation and apoptosis. This leads to an abnormal accumulation of keratinous debris, which is the characteristic feature of middle ear cholesteatoma [83].

An antiapoptotic compound known as galectin3 is a protein that may influence the events in cholesteatoma epithelium. Sheikholeslam-Zadeh et al. discovered that this protein promotes antiapoptotic effects and may thus be a protective mechanism against the intensified apoptosis present particularly in recurrent cholesteatoma [125].

Apoptosis in cholesteatoma is markedly intensified compared to skin. Hyperproliferation in cholesteatoma epithelium on the other hand counteracts apoptosis. The balance between hyperproliferation and apoptosis creates a higher cell turnover, which may be an important factor limiting the preneoplastic defect [39].

In a variety of cells, c-myc oncogene has been found to be highly linked to the control of growth and differentiation. The expression of c-myc was studied in cholesteatoma epithelium using a monoclonal antibody against the 67 kD c-myc gene product. Holly et al. found that the incidence of c-myc is significantly increased in nuclear staining of cholesteatoma epithelium [48]. Simultaneously, in cytoplasmic staining, c-myc was observed in both skin and cholesteatoma, with a stronger reactivity in the latter. These results also suggest a participation of the c-myc oncogene in the reported dysregulation of cholesteatoma epithelium. Kojima et al. observed that the proliferation of epidermal cells in cholesteatoma is not uncontrolled as it is in malignant tumors. They observed an increase in the proliferation rate and apoptotic cell death in cholesteatoma epidermis [61].

Annexin II has previously been discovered to be involved in DNA replication and metabolism, bone resorption and osteoclast formation. Immunoalkaline-phosphatase staining by Huang et al. selectively localized annexin II in the keratinocytes in the basal and spinous layers of cholesteatoma tissue [49]. In normal human skin, an-

nexin II was expressed mainly in the cytoplasmic membrane of keratinocytes in the basal layer without significant staining of the nuclei. However, annexin II was expressed in both the cytoplasmic membrane and the nuclei of the keratinocytes in basal and spinous layers of human cholesteatomas. This observation could be an indication for a physiologic role of annexin II in keratinocyte cell hyperproliferation during the development of human cholesteatoma [61].

Growth factors and cytokines

A characteristic sign of acquired cholesteatoma is the infiltration of the stroma with immune cells [5]. Many markers are responsible for the special behavior of acquired cholesteatoma, such as interleukin-1 α , tumor necrosis factor- α (TNF- α), intercellular adhesion molecule-1 (ICAM-1), lymphocyte functional antigen-1 (LFA-1), etc. Cholesteatoma matrix is characterized by a keratinocyte dysregulation with an aggressive growth that leads to the destruction of normal middle ear mucosa. The abnormal behavior of cholesteatoma epithelium seems to be induced by the presence of a heavy immune cell infiltrate releasing high amounts of different cytokines and growth factors. In normal epidermis, the restriction of keratinocyte proliferation to the basal cell layer is controlled by the local expression of transforming growth factor-alpha (TGF-alpha) and epidermal growth factor (EGF), and by the down regulation of epidermal growth factor-receptor (EGF-R) in the suprabasal cell layers. Several studies have reported defects in the regulation of the EGF-R system in cholesteatoma [18, 17, 135]. In contrast to normal external meatal skin, the high expression of the EGF-R in cholesteatoma is not confined to basal keratinocytes, but is also found in suprabasal keratinocytes in the stratum spinosum and granulosum. As many as 75% of the keratinocytes in cholesteatoma express EGF-R, whereas only 10% of the keratinocytes of the external meatal skin express EGF-R [14].

Psoriatic keratinocytes express the receptor for IFN γ (interferon-gamma) [150]. IFN γ increases the in vitro expression of EGFR. It may thus be a mediator for the overexpression of EGFR in psoriatic keratinocytes [41]. Both, IFN γ and IL-1 induce the expression of the intercellular adhesion molecule-1 (ICAM1), which is a member of the immunoglobulin superfamily and is the ligand of the lymphocyte function-associate antigen-1 (LFA1) [101]. In a study conducted by Ottaviani and co-workers, a cytokine and adhesion molecule pattern indicating an activation state has been observed in all cholesteatoma specimens. In the perimatrix, the endothelial cells express the inducible molecules ICAM1 and ELAM1 (endothelial-derived leukocyte adhesion molecule-1), which play a role in recruiting inflammatory cells and modulating immune response. The basal layer of cholesteatoma matrix is positive for ICAM1. The overexpression of IFN γ R in the matrix reinforces the hypothesis that the hyperproliferation of the epithelial cells of secondary cholesteatoma is mediated through lymphokines [94].

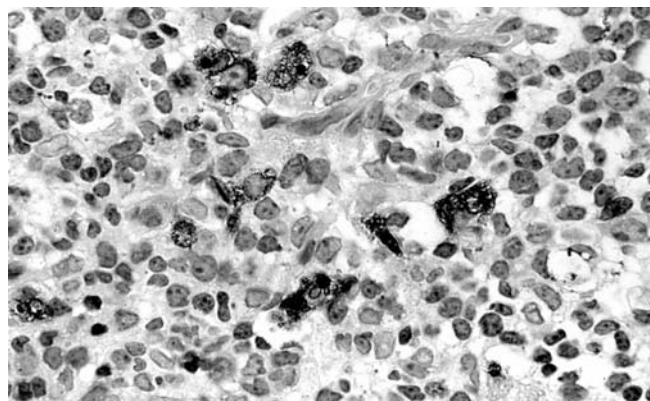


Fig. 10 Numerous macrophages within the inflamed perimatrix of cholesteatoma (APAAP immunohistochemistry, CD 68 staining, $\times 400$)

Further studies investigate the presence of interleukin-1 (IL-1), transforming growth factor-alpha (TGF-alpha) and epidermal growth factor (EGF) [120, 135]. The findings were compared to surrounding stroma with an enhanced immune cell infiltrate. Cholesteatoma epithelium showed a high staining intensity of IL-1, TGF-alpha and EGF-R. In contrast, middle ear mucosa did not show any positive reactions for the mentioned factors. The authors found a high concentration of lymphocytes and macrophages in the stroma surrounding cholesteatoma (Fig. 10). Most of these cells expressed TGF-alpha and IL-1, showing activation. Keratinocytes of the middle ear mucosa do not appear to react to the inflammatory stimuli released by cholesteatoma. This observation suggests important differences in the cell biological features of the keratinocytes in middle ear mucosa and cholesteatoma. Wright and co-workers investigated external and middle ear pathology in TGF-alpha-deficient animals [158]. The experiments were performed in the waved-1 mutant mouse, which is severely deficient in TGF-alpha: Morphologic changes of the external and middle ear were studied histologically at 2 weeks to 6.5 months of age. Abnormalities found in the mutants included epidermal hyperplasia of the external ear canal (EAC) and tympanic membrane (TM) and enlargement of specialized sebaceous glands adjacent to the cartilaginous EAC. Sebum and desquamated keratin progressively accumulated within the EAC, pushing the TM into the middle ear. However, the authors could not support the idea of TGF-alpha being an important player in the pathogenesis of cholesteatoma [158].

Besides, platelet-derived growth factor (PDGF), granulocyte-macrophage colony stimulating factor (GM-CSF) and cytokines such as tumor necrosis factor-alpha (TNF-alpha) seem to play a significant role in cholesteatoma pathogenesis [44, 49, 50, 161, 162] (Fig. 11). Investigations on growth factors in cholesteatoma by Ishibashi et al. revealed a significantly higher expression of keratinocyte growth factor (KGF) mRNA in cholesteatoma compared to normal skin, while no significant difference in KGFR mRNA expression was noted between cholesteatoma tissue and skin [51]. These findings suggest that ex-

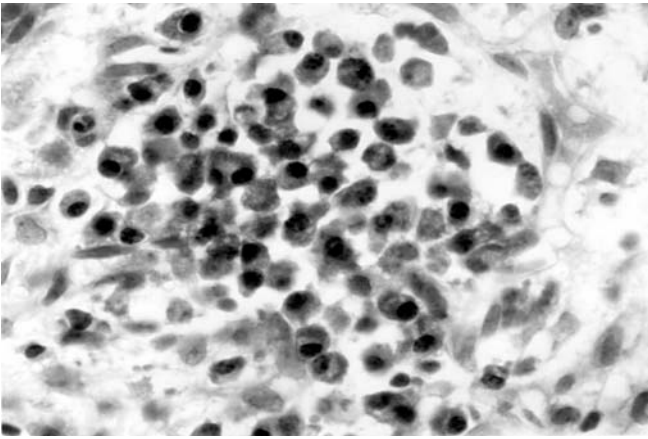


Fig. 11 Inflammatory cells express macrophage colony stimulating factor within the subepithelial connective tissue of cholesteatoma (MCSF, APPAP, $\times 640$)

cessive KGF synthesis may contribute to the hyperproliferative state in cholesteatoma.

Kato and co-workers recently reported that cholesteatoma cells produce IL-6 and TNF-alpha [55]. IL-6 and TNF-alpha produced in middle ear cholesteatoma might aggravate the disease by the wide range of their biological activities on the mucociliary system, cell proliferation and bone metabolism [55, 161, 162]. IL-1alpha and IL-8 from the cholesteatoma epithelium may be responsible for bone destruction and osteoclast activation. Certain substances relieved from the subepithelial granulation tissue seem to stimulate IL-1 alpha and IL-8 production in cholesteatoma [29].

Results published by Bujia and co-workers point out an over-expression of IL-1 accompanied by a decreased secretion of IL-1-receptor antagonist in middle ear cholesteatoma [21]. Furthermore, IL-1- receptor antagonist production was on a significantly lower level than total IL-1 production, resulting in a predominance of active IL-1. This may lead to an increased activation of osteoclasts and cause bone resorption [21].

Inflammatory reaction

Inflammation is always observed in cholesteatoma specimens. It represents a sequence of complex, interrelated events that occur as a response to tissue injury. Inflammation is induced by an immune response to different stimuli. An inflammatory response is essential for the repair and restoration of structural and functional integrity of damaged tissue. Irrespective of the nature of the occurring events, the wound healing process follows a predictable pattern, partly comparable to cholesteatoma development. The occurring events can be divided into early or acute inflammation, a proliferative phase characterized by fibroblast and endothelial cell proliferation and finally matrix synthesis and scar formation. In case of persistent inflammatory stimuli, such as in cholesteatoma, the response

may be chronic, resulting in pathological alteration of tissue behavior. Cholesteatoma pathogenesis can be interpreted as the reaction of migrating epithelium to an ongoing inflammation and bacterial infection, because cholesteatomas are usually associated with inflammatory reactions in the middle ear cavity, and inflammatory granulation tissue often appears along the invading epithelium in active cholesteatomas [71, 113].

An intense infiltration of the stroma of cholesteatoma by immunologically activated T-cells and macrophages indicates a chronic inflammatory response. Inflammation of the subepithelial connective tissue may contribute to the aberrant behavior of cholesteatoma epithelium [95]. The density of the macrophages in normal ear canal skin was found to be much higher in the upper dermis than in the lower dermis [95] (Fig. 10). A great number of phagocytic cells closely resembling dermal macrophages was found in the stroma of the cholesteatomas. These cells probably contribute to an active immune process [87]. Mast cell concentration in cholesteatomas is approximately three to seven times higher than in other tissues. Besides, 19–34% of the mast cells in cholesteatoma are found in the suprabasal layers of the squamous epithelium. This observation can only be made in grossly inflamed tympanic membrane specimens, but not in other control tissues, including minimally inflamed tympanic membranes. Albino and co-workers conclude from these results that mast cells may play a previously overlooked role in cholesteatoma pathology [7].

Extracellular matrix

The physiological functions of extracellular matrix (ECM) macromolecules such as collagen, fibronectin, integrins and glycosaminoglycans are linked with cell adhesion, cell migration, cell growth and differentiation. Quantitative and qualitative alterations of ECM may be induced by cytokines that modify connective tissue cell metabolism. Penetration of the submucosal space by middle ear infection and cell necrosis starts the wound healing cascade. In wound healing, connective tissue fibroblasts and macrophages play a pivotal role. Cytokines of wound healing promoting the re-epithelization of mucosal defects and scarring become effective on the intact squamous cell layer of the outer surface of the ear drum at the same time. Thus, a proliferation of the undamaged epithelial layer is induced. Persistence of the inflammation may cause permanent wound healing, proliferation of fibroblasts and epithelium in the perimatrix.

Dallari et al. investigated the expression of integrin adhesion molecules in primary acquired and recurrent cholesteatomas and compared it to common epidermal cysts and normal human skin [33]. In these experiments, cholesteatoma epithelium exhibited a markedly augmented expression of alpha v integrin subunit and a corresponding accumulation of vitronectin in the surrounding stroma. In contrast, the expression pattern of alpha 2 beta 1, alpha 3 beta 1 and alpha 6 beta 4 integrins as well as the distribution of laminin, collagen IV and fibronectin were similar in cholesteatomas, epidermal cysts and normal human skin [33].

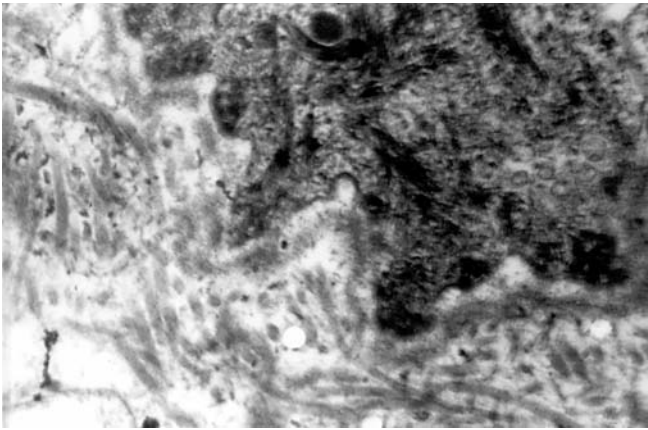


Fig. 12 Electron microscopy of cholesteatoma basement membrane zone revealing anchoring fibrils and scattered amorphous material of irregularly arranged collagen fibrils and fine fibrils. Basement membrane protrusions, reduplications and focal discontinuities are visible within the lamina densa ($\times 12,000$)

Consistent with other authors, we found immunohistochemical and ultrastructural alterations in the distribution of the basement membrane zone (BMZ) components collagen type IV, collagen type VII and fibronectin in human middle-ear cholesteatoma compared to auditory meatal skin [129, 137]. Collagen type IV immunoreactivity of skin and middle ear mucosa displays a continuous line in the BMZ, whereas in cholesteatoma focal discontinuities are frequently observed. In cholesteatoma, on the other hand, fibronectin immunoreactivity is markedly increased in the extrinsic BMZ and subepithelial connective tissue. The ultrastructural arrangement of the BMZ of cholesteatoma and skin is grossly the same. However, it exhibits distinct alterations of the lamina fibroreticularis and lamina densa (Fig. 12). Our results outline cholesteatoma as a disease with disturbed cell matrix interactions analogous to those of wound re-epithelization. Lang and co-workers also found a marked re-expression of tenascin. Fibronectin reactivity was observed as a continuous band along the epidermal-stromal junction, extending to the deeper stroma [66]. Additionally, in cases of intensified TGF-beta expression, beginning collagen fibril formation was observed in adjacent deeper stroma layers, indicating beginning stromal fibrosis. These observations suggest that TGF-beta may be involved in the stimulation of the synthesis of tenascin, fibronectin and collagen. Furthermore, the enhanced expression of tenascin and fibronectin provides evidence for a dysregulated cell-matrix interaction in the enhanced proliferative process of cholesteatoma formation.

Degradation of extracellular matrix (ECM) and proteolysis

As the ECM displays obvious alterations in cholesteatoma, it is reasonable to investigate degrading enzymes capable of disturbing the ECM. Among other enzymes, Abramson investigated collagenase in the subepithelial connective tis-

sue of cholesteatoma, granulation tissue from the middle ear and the dermis of canal skin [1, 2]. He could not prove the presence of collagenase in the keratin layer, epithelium or the epidermal appendages. Expression of collagenase enhanced by chronic inflammation attacks the intact collagen molecule, making it susceptible to further digestion by other proteases that are also products of inflammation. This process brings about resorption of connective tissue and bone. The proteolytic erosion of the temporal bone may play an important role in the process of cholesteatoma progression. Recently, a new family of proteolytic enzymes, the matrix-metalloproteinases (MMP), has been identified. MMPs seem to play a pivotal role in matrix and bone homeostasis as well as inflammatory osteolytic diseases, e.g., osteoarthritis and periodontitis. They are controlled in a sophisticated manner by specific inhibitors and activation cascades. Schönermark et al., Stark et al. and Banerjee and co-workers investigated the expression of MMPs and MMP-inhibitors in human cholesteatoma tissue [13, 14, 123, 129]. By immunocytochemistry and zymography, the expression of MMP-1, MMP-2, MMP-9 and MMP-3 was demonstrated to be strictly confined to the basal and suprabasal cell layer of cholesteatoma epithelium. Neutrophil collagenase showed a more disseminated expression in the epithelium and granulation tissue. The tissue inhibitor of MMPs, TIMP-1, could be detected only in very limited areas of the granulation tissue in a quite disseminated manner [129]. Interpreting these findings, a derailment of the normally tightly controlled MMP system in favor of proteolysis was postulated and found to play an active role in the molecular mechanism of cholesteatoma invasion into the temporal bone [123, 129].

Bone resorption

Bone resorption is stimulated by a variety of factors, including inflammation, local pressure, keratin and specific cytokines, such as interleukins (IL-1 α , IL-1 β and IL-6), interferon-beta (INF β) and parathyroid-hormone-related protein (PTHrP), which are all known to be released by cholesteatoma [3, 120] (Fig. 13). Inflammatory mediators such as cytokines, prostaglandins, NO (nitric oxide), growth factors and neurotransmitters are released in the chronic inflammatory process in cholesteatoma and may initiate osteoclast recruitment and bone resorption [52]. TNF- α (tumor necrosis factor alpha) is known to play a pivotal role in bone resorption [5]. It has a primary effect on osteoblasts, which then stimulate osteoclastic bone resorption [40]. Bone resorption comprises a sequence of events, beginning with the recruitment of mononuclear cells from bone marrow and multinucleation of these cells that then become osteoclasts (Fig. 14). These specialized cells are capable of resorbing the organic and anorganic matrix of bone. Activation of osteoclasts requires products of arachidonic acid metabolism, the previously mentioned cytokines as well as unknown factors. Bone resorption is also controlled by an obligatory relationship between local osteoblasts and osteoclasts [9, 24, 131]. Cinamon and al. observed that the



Fig. 13 Scanning electron photography showing an eroded incus removed during cholesteatoma surgery ($\times 2,000$)

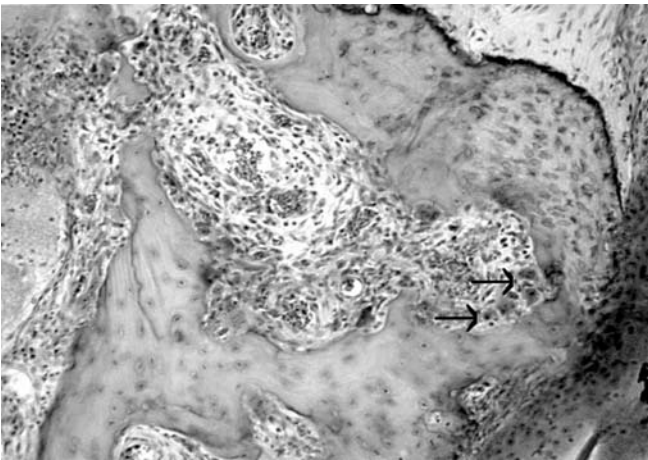


Fig. 14 Temporal bone histology of cholesteatoma revealing osteoclasts adjacent to the incus (HE, $\times 100$) with permission from Saumil N. Merchant, MD, Massachusetts Eye and Ear Infirmary, Boston, MA, USA

cholesteatoma-afflicted bone samples have an irregular border with a non-continuous periosteum. A loss of order and continuity of the cement lines between the bone lamellae was also observed. Most of the affected bones lack osteoblasts and osteocytes at sites of contact with cholesteatoma. Small, irregular eosophilic vesicles at the interface have also been observed [30]. Non-collagenous proteins such as osteopontin, bone sialoprotein, osteonectin and osteocalcin are mainly produced by osteoblasts and appear at different stages of the bone remodeling process. These proteins support cellular adhesion to the matrix, osteoclastic differentiation and regulation of the mineralization process, and besides have an affinity for bacterial adhesion [110, 111, 163].

The observation of sparse osteoblasts and a distinct difference in the content of non-collagenous proteins in the bone matrix adjacent to cholesteatoma may suggest an additional destructive impact through “down regulation” of osteoblasts [30]. Proteases such as plasminogen activators and matrix metalloproteinases (MMPs) have also been accused of playing a role in the degradation of bone matrix [23]. MMP-2 and MMP-9 both have been reported to contribute to bone remodeling by degrading basement membrane type IV collagens [74]. Presence of MMP-9 and MMP-2 has been observed in basal and suprabasal epithelial cell layers of cholesteatoma [121, 123]. The expression of MMP-9 (but not MMP-2) was increased in cholesteatoma compared to external ear canal skin samples. A possible explanation for the up-regulation of MMP-9 in cholesteatoma epithelial cells could be an immunological response to bacterial products stimulating macrophages to invade tissue and to produce cytokines. These cytokines may stimulate epithelial cells to produce proteases and cytokines [121]. However, further studies by the same authors showed none of the analyzed growth factors (EGF, TNF- α and TGF- β) increased significantly in correlation with MMP-9 activity values. This may be due to the fact that MMP up-regulation underlies a complex regulation that is not controlled by a single cytokine [122].

In 1980, Chole et al. discovered that Mongolian gerbils develop cholesteatoma with age [27]. They used this animal model to demonstrate that osteoclastic bone resorption is triggered by pressure [92]. Richardson et al. administered the bisphosphonate risedronate subcutaneously to neonatal mice, which resulted in a significant decrease of PTH-activated (parathyroid-hormone) protein calcium release in explanted calvaria in vitro [109]. Bisphosphonates resemble a promising new class of drugs in the treatment of bone resorption disorders, but require further investigations.

Angiogenesis

Angiogenesis is particularly important in a large number of normal and pathological processes, including wound healing and inflammation. The localization and distribution of angiogenic growth factors, basic fibroblast growth factor (FGF-2) and vascular endothelial growth factor (VEGF) in middle ear cholesteatoma has therefore been studied by many authors [81, 138]. Basic fibroblast growth factor plays a pivotal role in wound healing. It is chemotactic and mitogenic for fibroblasts, endothelial cells and keratinocytes. It can also stimulate the production of collagenase and plasminogen activators to enhance fibroblast proliferation and angiogenesis. FGF-2 could be demonstrated in cholesteatoma perimatrix. However, cholesteatoma matrix tissue without inflammation or any sign of wound healing has not expressed FGF-2 [81].

An enhanced expression of VEGF and FGF-2 in cholesteatoma in relation to middle ear mucosa and auditory meatal skin points out rapidly growing activated keratinocytes and endothelial cells. Vascularization within the perimatrix of cholesteatoma showed a five-fold increase

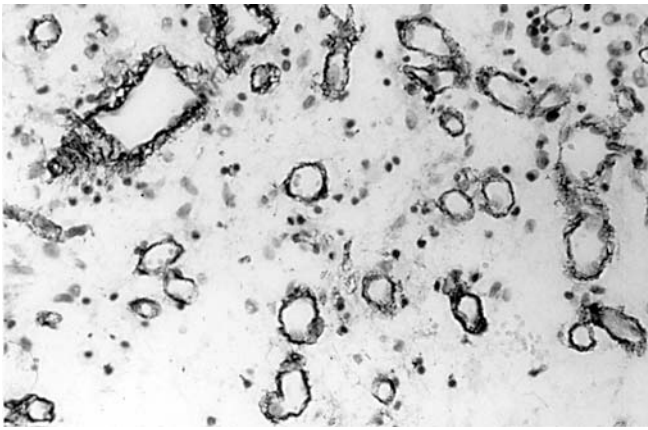


Fig. 15 Enhanced positive immunoreactivity for collagen type IV in vessels of cholesteatoma (collagen type IV, avidin-biotin-complex immunohistochemistry, $\times 64$)

compared to middle ear mucosa and a two-fold increase compared to skin (Fig. 15). We found a close relationship between the density of capillaries, degree of inflammation and expression of the investigated angiogenic factors [138]. Further studies confirm that with increasing degrees of inflammation, an enhanced vascularization within the perimatrix occurs. Because monocytes, macrophages and infiltrating leukocytes are known to produce cytokines such as FGF-2, VEGF, EGF, TGF- α , TGF- β , IL-1 and IL-6, angiogenesis might be induced by these inflammatory cells. Moreover, it could also be demonstrated that keratinocytes of cholesteatoma are able to release angiogenic factors. Proliferation of endothelial cells could be induced by the release of angiogenic factors from cholesteatoma matrix or by inflammatory cells of the subepithelial connective tissue. Additionally, the dual growth factor phenomenon of autocrine stimulation of cell proliferation and paracrine stimulation of the surrounding cells may play an essential role.

Angiogenesis enables and supports the migration of keratinocytes into the middle ear cavity [140]. As proliferating tissues such as middle ear cholesteatoma require an enhanced blood supply, angiogenesis appears to be a prerequisite for the expansion of cholesteatoma matrix within the middle ear.

Conclusions

The studies published to date support several suppositions concerning the pathogenesis of cholesteatomas. Cholesteatoma is a wound-healing process rather than a neoplastic lesion. Research to date does not indicate any genetic instability in cholesteatomas, which is a critical feature of malignant lesions. The activation of hyperproliferative cells in all epidermal layers of cholesteatoma potentially constitutes a response to both internal events as well as external stimuli represented by cytokines released by infiltrating inflammatory cells of the underlying subepithelial connective tissue.

The pathogenesis of cholesteatoma is certainly diverse, with different pathways leading to the same destructive lesion. There is no evidence for any obvious molecular or cellular differences among the various types of cholesteatomas (primary and secondary acquired or congenital). Approximately 30 years after his first publication on cholesteatoma in 1855, Virchow pointed out that he could not precisely explain the exact pathogenesis of this disease. Although there are several new ideas and results on its pathogenesis, Virchow's statement still remains valid today. Continued research should pay attention to the precise molecular and cellular dysfunction involved in the pathogenesis of cholesteatoma. This could ameliorate the understanding of this destructive middle ear lesion and be helpful in the development of new therapeutic strategies. To date, the only reasonable treatment for cholesteatoma is still surgery.

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