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REVIEW ARTICLE

Acquired cholesteatoma: summary of the cascade of molecular events

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Abstract

Background: Cholesteatoma is considered a benign, gradually expanding and destructive epithelial lesion of the temporal bone. The pathogenesis of different classifications of cholesteatoma is marked by similar underlying cellular and molecular processes. Stepwise explanations of the histopathogenesis have been described previously. The current paper focuses on expounding the molecular events of cholesteatoma.

Method and results: Cholesteatoma pathogenesis encompasses a complex network of signalling pathways during: epidermal hyperplasia, perimatrix–matrix interactions and mucosal disease. This paper presents a review of the molecular events driven by inflammatory mediators and enzymes during: cholesteatoma growth (cell proliferation and apoptosis); maintenance and deterioration (angiogenesis and hypoxia, oxidative stress and toxicity); and complications (bone erosion and hearing loss). The cascade of molecular events applicable to atelectasis and cholesteatoma that coexist with chronic otitis media and bone erosion as sequelae is summarised.

Conclusion: The role of lipids in this disease is relatively unexplored, but there is evidence in support of fatty acid role-players that needs confirmation. Future directions in lipid research to delineate molecular mechanisms are proposed.

Key words: Cholesteatoma; Ear; Middle; Inflammation; Biochemistry; Enzymes; Pathology

Literature review

A general review of cholesteatoma was conducted. Literature searches (using Medline and PubMed) included clinical and experimental work carried out over the past three decades. The literature selection was limited to English language articles. The review focused primarily on inflammatory mediators and enzyme activities. The objectives of the study were to: give an account of the cascade of molecular events involved in cholesteatoma and the complications thereof, and identify future research directions for a better understanding of cholesteatoma pathogenesis and deterioration, and bone erosion.

Introduction

Cholesteatoma is considered a benign, gradually expanding and destructive epithelial lesion of the temporal bone which results in erosion of adjacent bony structures, leading to various complications. Bone erosion of the ossicular chain and otic capsule (i.e. the bony labyrinth) may result in hearing loss, vestibular dysfunction, facial paralysis and intracranial complications.[1,2] The commonly accepted diagnostic classification of acquired cholesteatoma is that proposed by Tos (1993), which is based on site of origin.[3] Although acquired cholesteatomas may have different origins, they share similar cellular and molecular processes.

A previous review article on cholesteatoma (Louw, 2010) provided stepwise explanations of the histopathogenesis.[1] The current review article re-evaluated molecular events driven by inflammatory mediators and enzymes during the clinical course of acquired cholesteatoma (i.e. during hyperplasia and/or metaplasia, angiogenesis and hypoxia, oxidative stress and toxicity, and tissue degeneration and bone erosion).

Activities of lipid signalling pathways (i.e. fatty acid role-players and their enzyme activities) during hyperplasia and/or metaplasia (i.e. cell proliferation and anti-apoptosis), cell degeneration (i.e. pro-apoptosis and cell toxicity), and in complications (i.e. fatty acid substrates and enzyme activities that contribute to temporal bone erosion and nerve degeneration) are relatively unexplored. This paper summarises the cascade of molecular events applicable to atelectasis (with bone contact) and cholesteatoma (sacs or mass-like growths) that coexist with chronic otitis media and bone erosion as sequelae, as well as proposing future directions in lipid research.

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Cholesteatoma overview

Pathogenesis and aetiology

In principle, a cholesteatoma can present either as a sac that contains keratin with a surrounding keratinising squamous epithelial layer (i.e. matrix) and an adjacent subepithelial connective tissue layer with a bounding mucous cuboidal epithelial layer (i.e. perimatrix), or as a mass-like growth (i.e. haphazard growth with dispersed keratin deposits). Cholesteatoma pathogenesis can be explained on the premises of different triggers (trauma or disease) and different theories (migration, hyperplasia or metaplasia), or a combination thereof, as described in the review article of Louw (2010).[1]

The following text briefly summarises excerpts from Louw's (2010) paper. Triggers for cholesteatoma onset are diverse and may involve tympanic membrane trauma (perforation, displacement, retraction and invagination), or disease (chronic inflammation) and/or diseased mucosa of the tympanic cavity (i.e. otitis media with effusion). Taken together, the onset of acquired cholesteatoma can be marked by: epidermal cell migration through tympanic membrane perforations to form mucocutaneous junctions (i.e. pre-cholesteatoma conditions, without inflammation); epidermal cell hyperplasia (i.e. protruding masses through tympanic membrane perforations or from intact tympanic membranes or retraction pockets into the tympanic cavity, triggered by inflammation), with either sac-like growths (i.e. papillary cone and sinus formation patterns that fuse with keratin in the sac) or mass-like growths (i.e. haphazard growth patterns with dispersed keratin); and diseased mucosa of the tympanic cavity (i.e. mucosal metaplasia) with an impact on the tympanic membrane (i.e. cholesteatoma formation with secondary perforation of the tympanic membrane, triggered by inflammation).[1,2]

The main aetiological factors in cholesteatoma development are: long-term eustachian tube dysfunction and reduced middle-ear pressure (i.e. poor pneumatisation in the middle ear and/or mastoid process), resulting in tympanic membrane retraction pockets and invaginations (i.e. atelectasis and adhesive otitis media); and inflammatory conditions (i.e. chronic otitis media with effusion), resulting in negative middle-ear pressure and tympanic membrane retraction pockets and invaginations.[1,4]

Chronic inflammation and bacterial biofilms

Chronic inflammation plays an important role in the progression from pre-cholesteatoma conditions (i.e. mucocutaneous junctions, retraction pockets, atelectasis and adhesive otitis media) to cholesteatomas. The role of chronic inflammation in the clinical course of cholesteatoma is summarised in the following text. Bacterial otitis externa can trigger epidermal hyperplasia of the tympanic membrane, with epidermal projections into the tympanic cavity or epidermal ingrowths into the tympanic membrane. Persistent inflammation in tympanic membrane perimatrix and otitis media in the tympanic cavity are considered to be significant factors in cholesteatoma growth, expansion and invasion. Chronic otitis media is the culprit in diseased mucosa, and bouts of chronic infections contribute to cholesteatoma persistence and recurrence.[5–8]

Bacterial biofilms have been identified in cholesteatomas. Their actions can be described as either indirect signalling, wherein bacterial adherence to epithelial cells may lead to chronic infection and cell hyperproliferation, or direct signalling of bacterial products (endotoxins) that may elicit host immune responses.[9–11] *Pseudomonas aeruginosa* is an opportunistic bacterial pathogen capable of forming biofilms, and is the most common organism isolated from infected cholesteatomas.[12] Bacterial biofilms can be responsible for inflammatory reactions that may lead to chronic inflammation. This includes biofilms that are: trapped in the external acoustic meatus; present in lacunae, sinuses or sacs within cholesteatomas; or located in hollows of the tympanic cavity.[2,6]

Inflammatory mediators are produced by infiltrating immune cells (i.e. neutrophils, monocytes and lymphocytes) and local cells (such as keratinocytes and mast cells) in cholesteatomas.[13] During initiation of otitis media, the complement part of the immune system (consisting of multiple components that can interact to form enzymes) is activated by bacterial endotoxins; depletion of these endotoxins (such as the chemotactic agent C5a that activates immune cells) is known to cause chronic otitis media.[13] Research has revealed that bacterially infected cholesteatomas and chronic otitis media eventually disrupt the balance between bone formation (via osteoblasts) and bone resorption (via osteoclasts), leading to bone erosion.[14] Thus, middle-ear inflammatory mediators and toxins may result in ossicle erosion and hearing loss.[13]

Growth and deterioration

The more severe or chronic the inflammation, the greater the expected impact on cholesteatoma perimatrix–matrix interactions and layer thickness.[15] Thus, chronic and recurrent inflammations determine aggressive growth, and angiogenesis (i.e. microvessel formations) is a prerequisite for tissue maintenance.[16] In addition, chronic inflammatory conditions in the tympanic cavity (and mastoid cells) can lead to the accumulation of thick mucous masses between mucosal folds and within pouches, which can impede ventilation and drainage, with consequent hypopneumatisation and hypoxia.[17,18] Hypoxic conditions in the tympanic cavity can contribute to tympanic membrane retraction pockets and invaginations that may lead to cholesteatoma onset and growth. Hypoxia eventually leads to microvessel occlusion, with consequent apoptosis and deterioration of cholesteatoma tissue components.[19] Established cholesteatoma is marked by inflammatory granulation tissue, and fetid exudates and flakes of keratin are present.

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Molecular overview

Details of the molecular events that occur during the clinical course of cholesteatoma have been described previously; these are briefly depicted below. The research findings focus on growth (cellular overproduction and apoptosis), development (expansion and invasion) and destructive properties (bone erosion and hearing loss).

Cholesteatoma pathogenesis

Cholesteatoma is a growth disorder, and the epidermal growth factor receptor and keratinocyte growth factor receptor are up-regulated in cholesteatoma pathogenesis,[20–23] as is the ligand for the epidermal growth factor receptor, namely amphiregulin.[24] In addition, increased p63 expression and keratinocyte markers demonstrate uncoordinated hyperproliferation, migration and invasion properties.[25–28] The cell cycle inhibitory protein p27 is down-regulated,[29] and the ErbB-2 protein for accelerated cell proliferation and apoptosis is over-expressed.[30] The c-jun protein (associated with proliferation), c-myc protein (associated with differentiation) and p53 tumour suppressor gene (associated with apoptosis) are all up-regulated in cholesteatoma.[2,31,32] The ras protein (present in keratinocyte membranes) serves as a switch and activates mitogen-activated protein kinases for the transcription of proliferation genes (via c-jun present in keratinocyte nuclei).[33] Caspases-3, -8 and -14 signalling pathways play important roles in apoptotic or terminal differentiation of the matrix and accumulation of keratin debris.[34,35] Survival (anti-apoptotic) signalling pathways, such as those of phosphoinositide 3 kinase-AKT (protein kinase B) and phosphorylated extracellular-regulated kinases 1 and 2, are up-regulated in cholesteatoma perimatrix.[36,37] Enhanced vascular endothelial growth factor and fibroblast growth factor 2 expressions can stimulate the production of collagenase and plasminogen activators to enhance fibroblast proliferation and angiogenesis.[2,17,38] Derailment of the matrix metalloproteinases system plays an active role in cholesteatoma invasion of middle-ear spaces.[2,39]

It is suggested that enhanced human β-defensin 2 and 3 in cholesteatoma may induce an innate immune response.[40] It is also suggested that cell-mediated immunity has an important role in cholesteatoma development and its auto-destructive properties, based on a predominance of T lymphocytes (cluster of differentiation 3+) and histiocytes (cluster of differentiation 68+) in invasive cholesteatomas.[41] In addition, the enhanced expression of transforming growth factor β by fibroblasts during cholesteatoma pathogenesis supports the idea that the cholesteatoma activity is similar to that of a chronic wound healing process.[42] Transforming growth factor β is an important trigger for the production of extracellular matrix components (i.e. fibronectin, collagen, integrins and glycosaminoglycans) that are linked with cell adhesion, migration, growth and differentiation.[2]

Cholesteatoma complications

The role of osteoclasts in bone erosion has been firmly established.[43] Among molecular mediators implicated in bone resorption are: liposomal enzymes (such as acid hydrolyses) including matrix metalloproteinases and hexosaminidase A;[2,39,44] cytokines (i.e. interleukins (ILs) 1 and 6, and tumour necrosis factor α (TNF-α));[2,45] pro-inflammatory fatty acid metabolites (such as prostaglandin E2);[46] nuclear transcription factors (such as nuclear factor κβ);[47] bacteria (lipopolysaccharide);[48] and specific pH activities (i.e. demineralisation by acidic cholesteatomas).[2] Previous research indicated that IL-1 and IL-6 can stimulate hexosaminidase (specifically N-acetyl-β-D-hexosaminidase) activity that may lead to bone erosion.[44] More advanced research revealed that TNF-α, together with IL-1 and receptor activator of nuclear factor κβ ligand (ligands for activating nuclear factor κβ receptors), contribute to bone erosion.[49] The above-mentioned research findings correlated with bone erosion in the presence of chronic otitis media, with or without cholesteatoma. With respect to sensorineural hearing loss, it was previously suggested that during chronic suppurative otitis media (with or without cholesteatoma), the passage of bacterial endotoxins through the round window may cause damage to hair cells in the cochlear base.[50]

Lipid overview

The cascade of cellular, molecular and biochemical processes during the clinical course of cholesteatoma still needs to be properly defined. Foremost, the role of lipids (i.e. fatty acid role-players) during cholesteatoma pathogenesis and its complications need to be addressed, and is therefore briefly outlined below.

Membrane lipids in human cells and bacteria

In the bilipid membrane of a human cell, the phospholipid class and its subclasses are considered the most important lipids. Each class or subclass consists of a fatty acid composition or profile. Fatty acids play critical roles in membrane to nucleus gene regulation and immune responses, and are mediated by a complex array of signalling pathways. Upon stimulation, arachidonic acid is released from membrane phospholipids by phospholipase A2. Arachidonic acid is a source for the production of inflammatory metabolites via cyclo-oxygenase and lipoxygenase activities. Prostaglandin E2 production via cyclo-oxygenase-2 activity plays a crucial role during chronic otitis media. During inflammatory conditions, neutrophils (a major component of phagocytic cells) produce oxygen free radicals (oxidants) via inducible nitric oxide synthases, namely reactive oxygen species and nitric oxide (NO), which cause cell damage. Free radical scavengers (antioxidants), such as superoxide dismutase, glutathione peroxidase and catalase, have a protective effect against oxidative damage.[13,51,52] It was demonstrated that superoxide dismutase, glutathione peroxidase and catalase were significantly depleted in

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cholesteatomas, but there was no correlation with bone erosion.[53] Arachidonic acid is also a source for lipid peroxidation by free radicals, and the formation of harmful hydroperoxides reduces membrane fluidity and permeability, causing it to collapse. In addition, lipid hydroperoxides can decompose to yield a range of highly cytotoxic aldehydes, which can further perpetuate tissue damage. It was suggested that an increase in oxidative stress occurs in inflammatory conditions as a consequence of elevations in: phospholipase A2, inducible nitric oxide synthase, cyclo-oxygenase-2 and lipoxygenase activities. This leads to increased arachidonic acid release, faster arachidonic acid oxygenation, and increased reactive oxygen species and NO production, which triggers lipid peroxidation and cell damage.[51,52]

In bacteria, the outer layer of the bilipid membrane predominantly contains lipopolysaccharides, while the inner layer is composed mainly of phospholipids. Lipopolysaccharide essentially comprises a lipid A component, consisting mainly of saturated fatty acids with carbon chain lengths from 10 to 18, and a polysaccharide component that interacts with the environment as a defence mechanism.[54] Bacterial lipopolysaccharide stimulates the release of arachidonic acid and the production of prostaglandin E2 metabolites and NO radicals during chronic otitis media, and thereby increases mucous secretion and bone erosion. Recently, there has been progression in our understanding of endotoxin lipopolysaccharide as a potent inducer of inflammatory mediators in the pathogenesis of otitis media and sequelae.[13] Moreover, lipopolysaccharides can stimulate neutrophils for the release of antimicrobial myeloperoxidase under conditions of oxidative stress. Myeloperoxidase reacts with hydrogen peroxide in neutrophil phagolysosomes to form a complex for the oxidation of chloride to hypochlorous acid and other toxic products. In cholesteatoma, excessive secretion of reactive oxygen species and myeloperoxidase has been shown to be correlated with bone erosion.[55]

Fatty acid role-players during cell growth and apoptosis

Environmental factors (i.e. bacteria, viruses, fungi, free radicals and other foreign particles) can interfere with essential fatty acid metabolism (i.e. metabolism of the omega-6 and omega-3 fatty acid series) with linoleic acid and α-linolenic acid as substrates respectively, by inhibiting Δ6 and Δ5 desaturase activities.[56,57] Essential fatty acid metabolism of the omega-6 and omega-3 fatty acid series is illustrated in Figure 1. Under these circumstances, *de novo* fatty acid synthase activity is up-regulated during glucose metabolism, with overproduction of palmitic acid as the end product.[58] Based on research evidence, it is apparent that excessive linoleic acid, a ligand for peroxisome proliferator-activated receptor-γ, drives the mitogenetic signalling pathway, and that excessive palmitic acid, a ligand for peroxisome proliferator-activated receptor-δ, drives the apoptotic signalling pathway. However, the eventual over-expression of peroxisome proliferator-activated receptor-δ contributes to apoptotic resistance, which results in conditions of cellular overproduction (e.g. hyperplasia and metaplasia).[59] Notably, peroxisome proliferator-activated receptor-γ is reported to be up-regulated in cholesteatoma.[60] Moreover, linoleic acid peroxidation products, namely 9- and 13-hydroxyoctadecadienoic acid metabolites, which are activators and ligands for peroxisome proliferator-activated receptor-γ, have the capacity to induce apoptosis.[61] In retrospect, linoleic acid and arachidonic acid are excellent sources for free radical attack, oxidative stress and cell damage. Although the role of arachidonic acid in the clinical course of cholesteatomas has been firmly established, the role of linoleic acid still needs to be confirmed.

Bacterial interference with lipid metabolism may also cause accumulation of very long chain fatty acids (more than 22 carbon atoms), which requires peroxisomal β-oxidation by catalase activity. Peroxisomal β-oxidation is known to be inhibited by endotoxin lipopolysaccharides (i.e. the conversion of hydrogen peroxide into water and oxygen); cells with very long chain fatty acids are rendered toxic and they become apoptotic.[62] Not surprisingly, cholesteatoma debris was previously associated with bone erosion.[48] The accumulation of very long chain fatty acids in the epithelial cells of cholesteatoma and their toxicity needs to be confirmed or refuted. Of relevance to cholesteatoma is that newly formed tissue masses are maintained by angiogenesis and a constant supply of systemic dietary lipids. The latter explains why there is no exhaustion of lipid sources and why a cascade of events contributes to cholesteatoma growth and expansion, up to a point where hypoxia and microvessel occlusion lead to cholesteatoma degeneration. It has been revealed that under hypoxic conditions, the hypoxia inducible factor stimulates matrix metalloproteinases that cause cholesteatoma perimatrix degeneration.[63]

Fatty acid role-players during chronic inflammation and bone erosion

Central to inflammation are the inflammatory mediators produced by epithelial and endothelial cells, as well as immune infiltrates. These mediators either fight off infection or damage tissues, depending on overproduction, inhibition or an imbalance among family members. It is evident that lipopolysaccharides stimulate tumour necrosis factor α (TNF-α) production, which plays a pivotal role during infection in cholesteatoma and chronic otitis media conditions. Although mainly produced by macrophages, keratinocytes and epithelial cells also have the potential to induce TNF-α activity in cholesteatoma. Tumour necrosis factor α has the capacity to stimulate matrix metalloproteinases involved in cholesteatoma degradation and bone erosion. It can also induce excessive mucous secretion in chronic otitis media (with or without effusion) via mechanisms that stimulate inducible nitric

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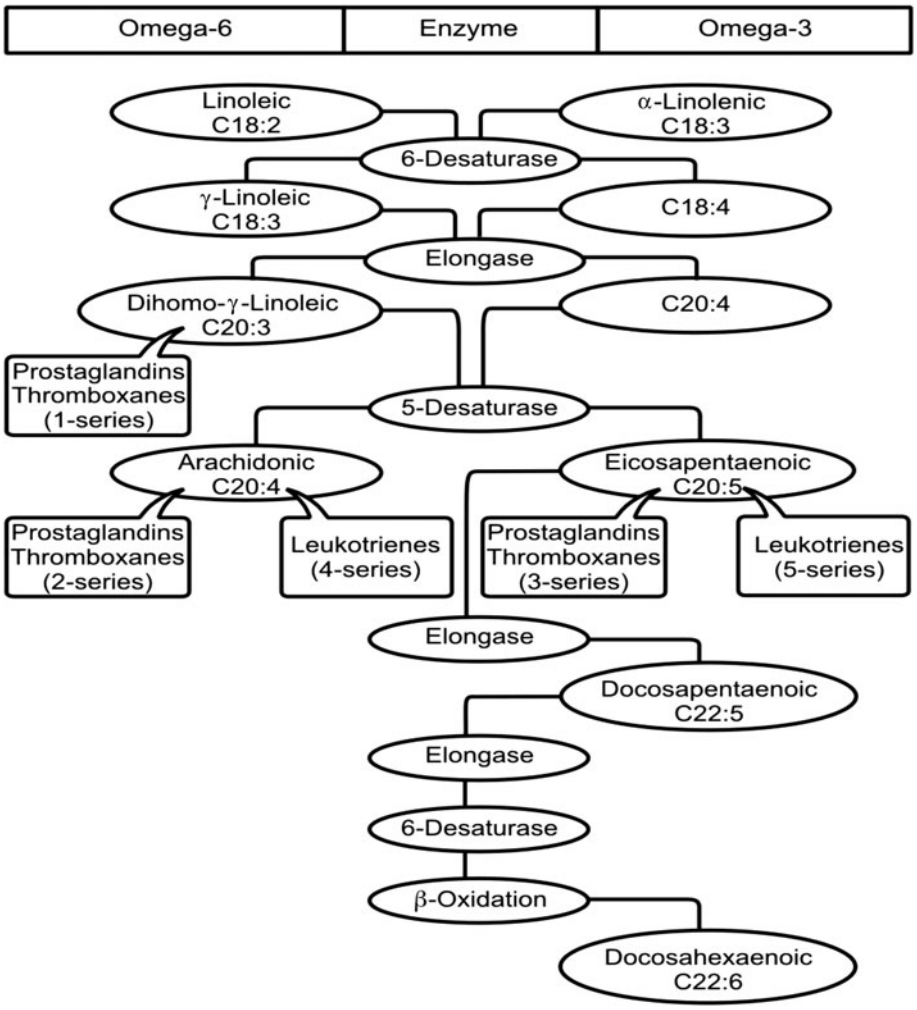

FIG. 1

Essential fatty acid metabolism of the omega-6 and 3 fatty acid series. Essential fatty acids include: linoleic acid and α-linolenic acid, which must be consumed by the diet; and γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid, which can be consumed by the diet or metabolised in the body. (Fatty acid nomenclature using the example 'C18:2': 18 = number of carbon atoms; 2 = number of double bonds.) Elongases are enzymes that elongate the carbon chain by two carbon units, desaturases introduce an additional double bond and β-oxidation refers to the shortening of the carbon chain by two units. Inflammatory metabolites include: prostaglandins and thromboxanes, which are produced via cyclo-oxygeneases; and leukotrienes, produced via lipoxygenases.

oxide synthases and toxic NO production, which may also lead to cholesteatoma degradation and bone erosion.[13]

Linoleic acid, α-linolenic acid and arachidonic acid are known to be potent natural ligands for peroxisome proliferator-activated receptor-γ. Furthermore, arachidonic acid and γ-linolenic acid metabolites are known to induce receptor activator of nuclear factor κβ ligand and osteoclastogenesis (via cyclo-oxygenase-2 activities).[13,64] However, the mechanisms involved still need elucidating.

Cascade of molecular events

It is proposed that lipopolysaccharides interfere with the first step in essential fatty acid metabolism, and that enhanced linoleic acid (and α-linolenic acid) stimulates epithelial hyperplasia, while enhanced fatty acid synthase activity induces palmitic acid overproduction with cell apoptotic resistance. Research has revealed that lipopolysaccharides stimulate prostaglandin E2 production (via arachidonic acid release) and tumour necrosis factor α (TNF-α) production (mostly via macrophage stimulation). The following text briefly outlines the network of interactions involved.

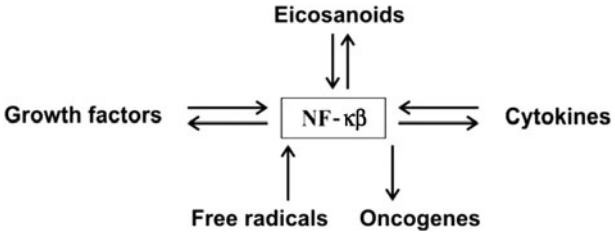

FIG. 2

Nuclear factor κβ feedback mechanisms. This transcription factor is activated by eicosanoids, cytokines, growth factors and free radicals, and in turn, stimulates eicosanoids, cytokines, growth factors and oncogenes (see Cascade of molecular events).

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TABLE I
CASCADE OF EVENTS DURING ATELECTASIS*

Disease condition
Eustachian tube dysfunction, poor pneumatisation, hypoxia & tympanic membrane invagination in contact with bone
Under hypoxic conditions, microvessel occlusion occurs & thinly stretched tympanic membrane collapses
Note: atelectasis often coexists with OM
Molecular events involved
Hypoxia stimulates HIF & induces MMP production, which causes tympanic membrane deterioration & bone erosion
Lipid peroxidation by radicals & production of toxic products (hydroperoxide & aldehydes) may also contribute to tympanic membrane deterioration
LPSs stimulate TNF-α production (in a wide variety of cells) & PGE2 production (via PLA activity & AA release), which are key inflammatory mediators of OM
LPS-induced OM results in mucoid MEE, & mucous secretions stimulate NOS activity & NO production which contributes to bone erosion

*With bone contact. OM = otitis media; HIF = hypoxia inducible factor; MMP = matrix metalloproteinase; LPS = lipopolysaccharide; TNF-α = tumour necrosis factor α; PGE2 = prostaglandin E2; PLA = phospholipase A; AA = arachidonic acid; MEE = middle-ear effusion; NOS = nitric oxide synthase; NO = nitric oxide

Lipopolysaccharides stimulate phospholipids for arachidonic acid release from membrane lipids. Arachidonic acid is a substrate for prostaglandin E2 production via cyclo-oxygenase-2 activity (stimulated by various factors, including enhanced saturated fatty acid levels and TNF-α). Enhanced prostaglandin E2 and TNF-α activate nuclear factor κβ expression that, in turn, stimulates prostaglandin E2 and TNF-α production. Under inflammatory conditions, enhanced reactive oxygen species and NO production (stimulated by phagocytes) enhance arachidonic acid (and linoleic acid) lipid peroxidation, and free radicals also activate nuclear factor κβ that, in turn, stimulates free radical production.[13,65] The feedback mechanisms of nuclear factor κβ are illustrated in Figure 2.

Previous cholesteatoma research has revealed that: NO synthase and toxic NO overproduction are involved in cholesteatoma degeneration and/or bone resorption;[66] matrix metalloproteinases, such as collagenases (stimulated by lipopolysaccharides and TNF-α), are

TABLE II
CASCADE OF EVENTS DURING CHOLESTEATOMA DEVELOPMENT*

Disease condition
Tympanic membrane trauma (perforations): epidermal hyperplasia (projections) triggered by inflammation (obits external), formation of mass-like chol (haphazard growths & dispersed keratin deposits)
Tympanic membrane disease (otitis externa): epidermal hyperplasia (growths) from tympanic membrane or retraction pockets, papillary cone formation & fusion with keratin deposits in lacunae, expansion into sac-like chol
Diseased mucosa of tympanic cavity (COM with or without effusion): impact on tympanic membrane with retraction pocket formations (or invaginations) & progression towards chol (with or without secondary tympanic membrane perforations)
Note: chol may coexist with or without COM
Molecular events involved
Excessive LA, including α-LA & AA (via Δ6d inhibition) & PA (via FAS stimulation) may stimulate hyperplasia (&/or metaplasia), & excessive VLCFAs (such as DHA) (via β-oxidation inhibition) are eventually cytotoxic to cells which may cause chol deterioration (based on lipid research)
Hypoxia stimulates HIF and induces MMP productions which cause chol deterioration & bone erosion
Inflammatory infiltrates (neutrophils) produce excessive NO (oxidant) via NOS stimulation & depletion of SOD, GP & CAT (antioxidants), which causes oxidative stress, chol deterioration & bone erosion
Neutrophil granules also release antimicrobial MPO, & toxic agents (such as HOCL) are released (via MPO-HOCL complex (within phagolysosomes)), which are associated with bone erosion
Lipid peroxidation & production of toxic products (such as hydroperoxide & aldehydes) may also contribute to chol deterioration
LPSs in chol & tympanic cavity biofilms cause: enhanced AA release via PLA stimulation & enhanced PGE2 production via COX-2 stimulation. COX-2 is a key inflammatory mediator of inflammation during chol matrix–perimatrix interactions & OM
LPSs also stimulate TNF-α production (in a wide variety of cells). TNF-α, PGE2 & oxygen radicals (NO) activate NF-κβ (central mediator of immune responses), which reactivates production of TNF-α & PGE2
LPS-induced OM results in mucoid MEE, & mucous secretions stimulate NO production (via enhanced NOS activity) which causes bone resorption
LPSs, TNF-α, IL-1 & IL-6 play prominent roles in pathogenesis of OM, AOM & COM
LPSs & TNF-α can: stimulate IL-1, IL-6 & HEX activity involved in bone erosion; stimulate IL-1 & RANKL (family member of TNF-α) involved in bone resorption; & inhibit peroxisome β-oxidation, whereby accumulation of VLCFAs may cause bone erosion
Chol debris can cause bone erosion
Note: degree of bone erosion depends on cumulative effects of events involved

*For both sacs or mass-like growths. Chol = cholesteatoma; COM = chronic otitis media; α- LA = alpha linolenic acid; AA = arachidonic acid; Δ6d = Δ6 desaturase; PA = palmitic acid; FAS = fatty acid synthase; VLCFA = very long chain fatty acid; DHA = docosahexaenoic acid; HIF = hypoxia inducible factor; MMP = metalloproteinase; NO = nitric oxide; NOS = nitric oxide synthase; SOD = superoxide dismutase; GP = glutathione peroxidase; CAT = catalase; MPO = myeloperoxidase; HOCL = hypochlorous acid; LPS = lipopolysaccharide; PLA = phospholipase A; PGE2 = prostaglandin E2; COX-2 = cyclo-oxygenase-2; OM = otitis media; TNF-α = tumour necrosis factor α; NF-κβ = nuclear factor κβ; MEE = middle-ear effusion; IL = interleukin; AOM = acute otitis media; HEX = hexosaminidase; RANKL = receptor activator of nuclear factor κβ ligand

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involved in perimatrix collagen degeneration and bone erosion;[39] and N-acetyl-β-D-hexosaminidase, together with interleukins (ILs) 1 and 6 (stimulated by lipopolysaccharides and TNF-α), are involved in bone resorption.[46] More advanced research has shown that: TNF-α, together with IL-1 and receptor activator of nuclear factor κβ ligand (a family member of TNF-α and ligand for osteoprotegerin), are involved in osteoclastogenesis and bone resorption.[13] In this paper, it is proposed that lipopolysaccharides and TNF-α inhibit catalases and peroxisomal β-oxidation of very long chain fatty acids, whereby these toxic (apoptotic) cells and/or their debris cause cholesteatoma degeneration, bone erosion and nerve degradation. This assumption is based on the rationale that the accumulation of very long chain fatty acids (tetracosanoic acid (C 24:00) and hexacosanoic acid (C 26:00)) in different tissues or biological fluids can lead to progressive demyelination of the peripheral nervous system.[67]

The cascade of molecular events applicable to atelectasis (with bone contact) and cholesteatomas (sacs or mass-like growths), which co-exist with chronic otitis media and bone erosion as sequelae, are depicted in Tables I and II. Evidently, the degree of bone erosion and/or resorption depends on a cumulative effect of the events involved.

Future directions

It is clear from the preceding information on lipids that there is a need to construct a complete lipid model that can serve as a sound foundation for further research. The model would consist of: lipid classes, such as total lipids, neutral lipids and membrane lipids (i.e. phospholipids, triglycerides and cholesterol esters); phospholipid subclasses (namely phosphatidyletanolamine, phosphatidylserine, phosphatidylcholine and phosphatidylinositol); and free fatty acids. The question that remains is: to what extent do fatty acids, direct or indirect, contribute to cholesteatoma pathogenesis (growth and deterioration) and complications (bone destruction and related neuropathies)? Investigation into the role of lipids is warranted. This should comprise advanced lipid analyses (lipidomics), and special reference should be given to fatty acid role-players and enzyme activities that occur during pathogenesis and complications of cholesteatoma.

Conclusion

The phenomenon of cholesteatoma has been extensively researched. There are on-going attempts to unravel all the mechanisms involved at a biochemical level. It is clear from the above discussion that there is also a lipid research field open for exploration.

References

1 Louw L. Acquired cholesteatoma pathogenesis: stepwise explanations. *J Laryngol Otol* 2010;**124**:587–93
2 Olszewska E, Wagner M, Bernal-Sprekelson M, Ebmeyer J, Dazert S, Hildmann H *et al*. Etiopathogenesis of cholesteatoma. *Eur Arch Otorhinolaryngol* 2004;**261**:6–24
3 Tos M. *Manual of Middle Ear Surgery*. New York: Thieme, 1993
4 Seibert JW, Danner CJ. Eustachian tube function and the middle ear. *Otolaryngol Clin North Am* 2006;**39**:1221–35
5 Chole RA, Sudhoff HH. Chronic otitis media, mastoiditis and petrositis. In Cummings *Otolaryngology: Head and Neck Surgery*, 4th edn: Mosby Elsevier, 2005;**4**:2988–3003
6 Sudhoff H, Tos M. Pathogenesis of sinus cholesteatoma. *Eur Arch Otorhinolaryngol* 2007;**264**:137–43
7 Mishiro Y, Sakagami M, Kitahara T, Kondoh K, Okumura S. The investigation of the recurrence rate of cholesteatoma using Kaplein-Meier survival analysis. *Otol Neurotol* 2008;**29**:803–6
8 Mustafa A, Heta A, Kastrai B, Dreshaj SH. Complications of chronic otitis media with cholesteatoma during a 10-year period in Kosovo. *Eur Arch Otorhinolaryngol* 2008;**265**:1477–82
9 Chole RA, Faddis BT. Evidence for microbial biofilms in cholesteatomas. *Arch Otolaryngol Head Neck Surg* 2002;**128**:1129–33
10 Post JC, Hiller NL, Nistico L, Stoodley P, Ehrlich GD. The role of biofilms in otolaryngologic infections: update 2007. *Curr Opin Otolaryngol Head Neck Surg* 2007;**15**:347–51
11 Macassey E, Dawes P. Biofilms and their role in otorhinolaryngological disease. *J Laryngol Otol* 2008;**122**:1273–8
12 Wang E, Jung J, Nason R, Pashia M, Chole R. Characterization of otopathogenic Pseudomonas aeruginosa: a biofilm phenotype. *Arch Otolaryngol Head Neck Surg* 2005;**131**:983–9
13 Junh SK, Jung M-K, Hoffman MD, Drew BR, Preciado DA, Sausen NJ *et al*. The role of inflammatory mediators in the pathogenesis of otitis media and sequelae. *Clin Exp Otorhinolaryngol* 2008;**1**:117–38
14 Peek FAW, Huisman M, Berckmans RJ, Sturk A, van Loon J, Grote JJ. Lipopolysaccharide concentration and bone resorption in cholesteatoma. *Otol Neurotol* 2003;**24**:709–13
15 Dornelles C, da Costa SS, Meurer L, Schweiger C. Correlation of cholesteatomas perimatrix thickness with patient's age. *Rev Bras J Otorrinolaringol* 2005;**6**:792–7
16 Nagai T, Suganuma T, Ide S, Shimoda H, Kato S. Confirmation of mucin in lymphatic vessels of acquired cholesteatoma. *Eur Arch Otorhinolaryngol* 2006;**263**:361–4
17 Sudhoff H, Dazert S, Gonzales AM, Borkowski G, Park SY, Baird A *et al*. Angiogenesis and angiogenic growth factors in middle ear cholesteatoma. *Am J Otol* 2000;**21**:793–8
18 Adunka O, Gstoettner W, Knecht R, Kiener AC. Expression of hypoxia inducible factor 1 alpha and Von Hippel Lindau protein in human middle ear cholesteatoma. *Laryngoscope* 2003;**113**:1210–15
19 Olszewska E, Chodynicki S, Chyczewski L. Apoptosis in the pathogenesis of cholesteatoma in adults. *Eur Arch Otorhinolaryngol* 2006;**263**:409–13
20 Alves AL, Pereira CSB, Carvalho M de FP, Fregnani JHTG, Ribeiro FQ. EGFR expression in acquired middle ear cholesteatoma in children and adults. *Eur J Pediatr* 2012;**171**:307–10
21 Barbara M, Raffa S, Mure C, Manni V, Ronchetti F, Monini S *et al*. Keratinocyte growth factor receptor (KGF-R) in cholesteatoma tissue. *Acta Otolaryngol* 2008;**128**:360–4
22 Yamamoto-Fukuda T, Takahasi H, Koji T. Expression of keratinocyte growth factor and its receptor in a middle ear cavity problem. *Int J Pediatr Otorhinolaryngol* 2012;**76**:76–81
23 Jin BJ, Min HJ, Jeong JH, Park CW, Lee SH. Expression of EGFR and microvessel density in middle ear cholesteatoma. *Clin Exp Otorhinolaryngol* 2011;**4**:67–71
24 Macias MP, Gerkin RD, Macias JD. Increased amphiregulin expression as a biomarker of cholesteatoma activity. *Laryngoscope* 2010;**120**:2258–63
25 Kucczkowski J, Pawelczyk T, Bakowska A, Narozny W, Mikaszewski B. Expression of patterns of Ki-67 and telomerase activity in middle ear cholesteatoma. *Otol Neurotol* 2007;**28**:204–7
26 Olszewska E, Lautermann J, Koc C, Schwaab M, Dazert S, Hildmann H *et al*. Cytokeratin expression pattern in congenital and acquired pediatric cholesteatoma. *Eur Arch Otorhinolaryngol* 2005;**262**:731–6
27 Olszewska E, Sudhoff H. Comparative cytokeratin distribution patterns in cholesteatoma epithelium. *Histol Histopathol* 2007;**22**:37–42
28 Park HR, Min SK, Min K, Jun SY, Seo J, Kim HJ. Increased expression of p63 and survivin in cholesteatomas. *Acta Otolaryngol* 2009;**129**:268–72

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29 Kuczkowski J, Bakowska J, Pawelczyk T, Narozny W, Mikaszewski B. Cell cycle inhibitory protein p27 in human middle ear cholesteatoma. *ORL J Otorhinolaryngol Relat Spec* 2006;**68**:296–301
30 Sakamoto T, Kondo K, Yamasodba T, Suzuki M, Sugasawa M, Kaga K. Over-expression of ErbB-2 protein in middle ear cholesteatomas. *Laryngoscope* 2004;**114**:1988–91
31 Osturk K, Yildirim MS, Ascar H, Cenik Z, Keles B. Evaluation of c-myc status in primary acquired cholesteatoma by using fluorescence in situ hybridization techniques. *Otol Neurotol* 2006; **27**:588–91
32 Motamed M, Powe D, Kendall C, Birchall JP, Banerjee AR. p53 Expression and keratinocyte hyperproliferation in middle ear cholesteatoma. *Clin Otolaryngol Allied Sci* 2002;**27**:505–8
33 Huang CC, Chen CT, Huang TS, Shinoda H. Mediation of signal transduction in keratinocytes of human middle ear cholesteatoma by ras protein. *Eur Arch Otorhinolaryngol* 1996;**253**:385–9
34 Miyao M, Shinoda H, Takahashi S. Caspase-3, caspase-8 and nuclear factor-kappa beta expression in human cholesteatoma. *Otol Neurotol* 2006;**27**:8–1
35 Jung MH, Lee JH, Cho JG, Jung HH, Hwang SJ, Chae SW. Expressions of caspase-14 in human middle ear cholesteatoma. *Laryngoscope* 2008;**118**:1047–50
36 Huisman MA, De Heer E, Grote JJ. Terminal differentiation and mitogen-activated protein kinase signaling in human cholesteatoma epithelium. *Otol Neurotol* 2006;**27**:422–6
37 Huisman MA, De Heer E, Grote JJ. Survival signaling and terminal differentiation in cholesteatoma epithelium. *Acta Otolaryngol* 2007;**127**:424–9
38 Niam R, Chang RC, Sadick H, Bayerl C, Bran G, Hormann K. Effect of vascular endothelial growth factor on fibroblasts from external auditory canal cholesteatoma. *Arch Med Res* 2005;**36**: 518–23
39 Morales SD, Penido N de O, da Silva NID, Stavale JN, Guilherme A, Fukuda Y. Matrix metalloproteinase 2: an important genetic marker for cholesteatomas. *Braz J Otorhinolaryngol* 2007;**73**:51–7
40 Song JJ, Chae SW, Woo JS, Lee HM, Jung HH, Hwang SJ. Differential expression of human beta defensin 2 and human beta defensin 3 in human middle ear cholesteatoma. *Ann Otol Rhinol Laryngol* 2007;**116**:235–40
41 Hussein MRA, Sayed RM, Abu-Dief EE. Immune cell profile in invasive cholesteatomas: preliminary findings. *Exp Mol Pathol* 2010;**88**:316–23
42 Huisman MA, de Heer E, Dijke PT, Grote JJ. Transforming growth factor beta and wound healing in human cholesteatoma. *Laryngoscope* 2007;**118**:94–8
43 Jung JY, Chole RA. Bone resorption in chronic otitis media: the role of the osteoclast. *ORL J Otorhinolaryngol Relat Spec* 2002; **64**:95–107
44 Olszweska E, Borzym-Kluczyk M, Olszweska S, Rogowski M, Zwierz K. Hexosaminidase as a new potential marker for middle ear cholesteatoma. *Clin Biochem* 2006;**39**:1088–90
45 Vitale RF, Ribeiro F de AQ. The role of tumor necrosis factor-alpha in bone resorption present in middle ear cholesteatoma. *Bras J Otorrinolaringol* 2007;**73**:123–7
46 Yuan J, Akiyama M, Nakahama K-I, Sato T, Uematsu H, Morita I. The effects of polyunsaturated fatty acids and their metabolites on osteoclastogenesis in vitro. *Prostaglandins Other Lipid Mediat* 2010;**92**:85–90
47 Byun JY, Yune TY, Lee JY, Yeo SG, Park MS. Expression of CYLD and NF-κB in human cholesteatoma epithelium. *Mediators Inflamm* 2010;**2010**:796315. Epub 2010 Apr 21
48 Iino Y, Toriyama M, Ogawa H, Kawakami M. Cholesteatoma debris as an activator of human monocytes. *Acta Otolaryngol (Stockh)* 1990;**110**:410–15
49 Nason R, Jung JY, Chole RA. Lipopolysaccharide-induced osteoclastogenesis from mononuclear precursors: a mechanism for osteolysis in chronic otitis. *J Assoc Res Otolaryngol* 2009; **10**:151–60
50 Hellstrom S, Eriksson PQ, Yoon YJ, Johansson U. Interactions between the middle ear and the inner ear: bacterial products. *Ann N Y Acad Sci* 1997;**830**:110–19
51 Aggarwal BB, Shishodia S, Sandur SK, Pandey MK, Sethi G. Inflammation and cancer: how hot is the link? *Biochem Pharmacol* 2006;**72**:1605–21
52 Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser MJ. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007;**39**:44–84
53 Eskiizmir G, Yuceturk AV, Onur E, Var A, Temiz P. The imbalance of enzymatic antioxidants in cholesteatoma. *Acta Otolaryngol* 2009;**129**:1187–91
54 Online textbook of bacteriology. In: http://textbookofbacteriology.net [30 January 2012]
55 Celebi O, Paksoy M, Aydin S, Sanh A, Tasdemir O, Gull AE. Myeloperoxydase activity in the pathogenesis of cholesteatoma. *Indian J Otolaryngol Head Neck Surg* 2010;**62**:32–5
56 Horrobin DF. Medical uses of essential fatty acids. *Vet Dermatol* 1994;**4**:161–6
57 Das UN. Essential fatty acids: a review. *Curr Pharm Biotechnol* 2006;**7**:467–82
58 Shah US, Dhir R, Golli SM, Chandran UR, Lewis D, Acquafondata M *et al*. Fatty acid synthase gene overexpression and copy number gain in prostate adenocarcinoma. *Hum Pathol* 2006;**37**:401–9
59 Martinasso G, Oraldi G, Trombetta A, Maggiora M, Bertetto M. Involvement of PPARs in cell proliferation and apoptosis in human colon cancer specimens and in normal and cancer cell lines. *PPAR Res* 2007;**2007**:93416
60 Hwang SJ, Kang HJ, Song JJ, Kang JS, Woo JS, Chae SW *et al*. Up-regulation of peroxidase proliferator-activated receptor gamma in cholesteatoma. *Laryngoscope* 2006;**116**:58–61
61 Niki E, Yoshida Y, Saito Y, Noguchi N. Lipid peroxidation: mechanisms, inhibition, and biological effects. *Biochem Biophys Res Commun* 2005;**338**:668–76
62 Khan M, Contreras M, Sing I. Endotoxin-induced alterations of lipid and fatty acid compositions in rat liver peroxisomes. *J Endotoxin Res* 2000;**6**:41–50
63 Knowles HJ, Cleton-Jansen A-M, Korsching E, Athanasou NA. Hypoxia-inducible factor regulates osteoclast-mediated bone resorption: role of angiopoietin-like 4. *FASEB J* 2010;**24**:4648–59
64 O'Shea M, Bassagaya-Riera J, Mohede IC. Immunomodulatory properties of conjugated linoleic acid. *Am J Clin Nutr* 2004;**79**: 1199S–1206S
65 Aggarwal BB. Nuclear factor-kappa B: a transcription factor for all seasons. *Expert Opin Ther Targets* 2007;**11**:109–10
66 Jung JY, Lin AC, Ramos LM, Faddis BT, Chole RA. Nitric oxide synthase I mediates osteoclast activity in vitro and in vivo. *J Cell Biochem* 2003;**89**:613–21
67 Deon M, Garcia MP, Sitta A, Barschak AG, Coelho M, Graziela O *et al*. Hexacosanoic acid and docosanoic acids plasma levels in patients with cerebral childhood and asymptomatic X-linked adrenoleukodystrophy: Lorenzo's effect. *Metab Brain Dis* 2008;**23**:43–9

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