The role of cytokines in acquired middle ear cholesteatoma: literature review

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Summary

The present study conducted a critical analysis of the literature regarding the role of cytokines in acquired cholesteatomas. Middle ear cholesteatomas are characterized by the presence of stratified squamous epithelium in this cavity revealing highly invasive properties evolving bone destruction and subsequent complications. Cytokines are low molecular weight-glycoproteins able to act in cellular intercommunication. They are important to stimulation and suppression of immune response-related events, triggering and coordinating inflammatory response, as well as wound healing and tissue remodeling. In cholesteatoma, reported cytokines and growth factors were as follows: IL-1, IL-6, IL-8, TNF-α, TGF-α, TGF-β, EGF and KGF. A synergism among different cytokines should occur to generate the aggressive characteristics of cholesteatoma.

INTRODUCTION

Middle ear cholesteatoma disease is histologically typified by the presence of squamous stratified keratinized epithelium at the site, which is typically lined by ciliated pseudo-stratified epithelium.

Cholesteatoma has some migratory and lytic characteristics, which may result in bone erosion of both ossicular chain and mastoid cells resulting in intra and extra-cranial complications.

Several authors have studied cholesteatoma trying to discover the etiopathogenesis of the disease that remains still controversial. In many of such studies those that used immunohistochemical methods provided in depth analysis of cytokines and their role in the etiopathogenesis of cholesteatoma.

These immunohistochemical studies brought some improvement to the diagnosis of several diseases providing identification of specific proteins of a given tissue through antigen-antibody reaction, which is not possible to achieve with conventional histological techniques, demonstrating that inflammation releases cytokines and that they stimulate the inflammatory process, triggering the cascade reaction. Cytokines are proteins produced by the cells in response to an inflammatory process and they act by modifying their characteristics and those from the surrounding cells. Many of such characteristics have already been studied and defined, some of them cause vasodilation, other cause osteolysis, mast cell and/or epithelial cell migration and formation of granular tissue. This interaction among cytokines is at the same time the cause and the effect of cholesteatoma aggressive behavior.

Middle ear cholesteatoma are part of ENT professionals' routine and many of its characteristics regarding etiopathogenesis and aggressiveness are still controversial. The objective of this study was to perform literature review and critical analysis related to the expression of several cytokines in cholesteatoma, identifying those which are responsible for its different behavior.

LITERATURE REVIEW

Cholesteatoma is a very common disease in our setting, but a rare disorder in other countries accounting for 0.1% - 0.5% of all ear diseases. Male-female ratio is 1.2:1.0 with age ranging from 3 to 70 years old.[1]

Histologically, the cholesteatoma epithelium is similar to that of the normal epidermis, formed by four conventional layers of epidermic tissue (stratum germinativum (SG), stratum spinosum (SS), stratum granulosum (SGR), stratum corneum). The epithelium of the cholesteatoma is known as the matrix, and loose subepithelial connective tissue containing collagen fibers, fibroblasts and inflammatory cells is known as perimatrix of the cholesteatoma[2].

Most of the inflammatory infiltrate cells are lymphocytes and macrophage, both in immunologically active state, suggesting that cholesteatoma inflammation is an immune-mediated process[3].

Cholesteatoma has hyperproliferative characteristics that are related to cytokeratin (CK) and Ki-67[4,5] presence. Cytokeratins are protein filaments of the cyto-skeleton of epithelial cells. Since the external auditory canal (EAC) and the cholesteatoma are histologically similar to epidermis tissues, they have cytokeratins 1, 2, 5, 10 and 14. Curiously, there is one cytokeratin of cholesteatoma that does not appear in middle ear mucosa nor in the epithelium of most parts of the EAC, which is CK 16. This cytokeratin is characteristic of epithelial cells in hyperproliferative stage. It is present in all epidermic benign hyperproliferation affections such as psoriasis, actinic keratosis, seborrheic dermatitis and warts, or malignant affections such as squamous cell carcinoma. It is also present in zones subjected to pressure (feet and digital pulp) and in the pavement epithelium of follicles. It appears in suprabasal layers of the cholesteatoma matrix, showing that this epithelium, regardless of its histological similarity to normal skin, has a hyperproliferative behavior[4]. The Ki-67 is a nuclear antigen that appears in cell multiplication stages (G1, S, G2 M phases of the cell cycle). The Ki-67 presence in all layers of the cholesteatoma epithelium gives it hyperproliferative characteristics[5].

The cytokines

The cytokines include a large number low-molecular weight glycoproteins (lower than 80 kD) that act in cell intercommunication. They can be secreted and/or expressed in cell membranes of the extracellular matrix[6].

One of the most important aspects of these proteins is their broad spectrum and effect potential. They can be produced by any body cell, except by erythrocytes. They are essential in immune response stimulation and suppression events, triggering or coordinating inflammatory response, including healing and remodeling tissue processes.[6]

Currently the word cytokine is used as a generic name for a diverse group of proteins and soluble polypeptides that act as humoral regulators in small concentrations[6].

The cytokines are also known as lymphokine, monokine, immunetransmitters, immunocytokines, chemokines, interleukins and interferons[7].

Natural immunity, effector cytokines are mainly mononuclear phagocytes and are typically known as monokines. Most of the cytokines of specific immunity are produced by T-activated lymphocytes and are commonly known as lymphokines[7].

Some cytokines share the ability to stimulate chemokinesis and the oriented move (chemotaxia) and have been collectively known as "chemokines", a contraction of chemotactics cytokines[7].

An important hypothesis generated in the 70's was that cytokines were mainly synthesized by leukocytes and acted primarily over other leukocytes and, therefore, could be called interleukins (IL)[7].

Different cytokines share the same properties, known as general properties described as follows:

1. Cytokines act over many different types of cells. This property is known as pleiotropism.
2. The effect of cytokines is usually redundant. Many functions originally attributed to a cytokine have been proven to be properties that are shared among other different cytokines. This finding has been confirmed by a study carried out in mice subjected to a gene elimination process and that did not have specific cytokine genes, but still demonstrated subtle immune response abnormalities.
3. Cytokines usually influence the effect of other cytokines. Two cytokines may interact antagonically, producing additive effects or, in some cases producing effects that are stronger or even more peculiar than those anticipated, a type of interaction typically known as synergism.
4. In general, cytokines influence the synthesis of other cytokines triggering a cascade effect in which a second or third cytokine may mediate biological effects of the first cytokine. The capacity of the cytokine to maximize and suppress the production of others may provide important positive and negative regulatory mechanisms for immune and inflammatory responses.
5. Similarly to other polypeptide hormones, cytokines initiate their effect by binding with specific receptors of the target-cell membrane. Cytokine receptors usually demonstrate high receptor-binding affinity. Therefore, only small amounts of cytokine are required to trigger a biological effect.
6. Cytokine effects may be local or systemic. The target-cell could be the same cell that secrets the cytokine (autocrine and intracellular effect) or a neighboring cell (paracrine or intercellular effect). If cytokines are produced in large amounts they may enter the bloodstream and act on targets distant from production site (endocrine effect).
7. Cytokines act as cell division regulators for many target cells, or as growth factors.

Some immunologists proposed that cytokines should be grouped together with growth factors of the epithelia or mesenchyma cells in a larger functional group of regulating polypeptidic molecules [6]. Other authors, however, continued to differentiate those molecules whose primary effect is to mediate host defense (that is, cytokines) of the molecules whose primary role is to repair tissue (that is, growth factors of epithelial and mesenchymal cells)[7].

Cholesteatoma cytokines

In depth data regarding the most studied cytokines in acquired cholesteatoma will be discussed as follows:

Interleukin 1 (IL1) is mainly produced by macrophages, and also by endothelial, epithelial Langerhans cells, lymphocyte T, B and NK, fibroblasts, mesangial and glia cells. IL1 is mediator of inflammatory response in natural immunity and stimulates bone reabsorption, increasing the number of osteoclast precursor cells. It also stimulates fibroblasts and osteoclasts to produce prostaglandin and collagenase. It is synthesized under two forms: IL-1α and IL-1β[7]. Several authors described IL1 in acquired cholesteatoma [8-13].

Interleukin 6 (IL-6) is a cytokine of 26 kD, synthesized by many different types of macrophages (primarily), lymphocyte T and B, fibroblasts, cells of bone marrow stroma and epithelial and endothelial cells. It stimulates T cell proliferation, the activation of a natural mechanism of cellular death and cytotoxicity [7]. Many authors studied IL-6 in acquired cholesteatoma. [8,14,15].

Tumor necrosis factor-alpha (TNF-α), also known as cachectin, is mainly produced by macrophages, but can also be released by lymphocyte and mast cells. It induces collagenase and prostaglandin production and is a chemotactic to inflammatory cells. TNF-α regulates the synthesis of other cytokines such as IL-6 and IL-1[6,7].Several authors have studied them in acquired cholesteatoma [8,12,13,15-18].

Transforming Growth Factor-Beta (TGF-b) is produced by endothelial cells, macrophages, neutrophils, platelets and lymphocytes T and B. Regarding inflammatory modulation, its effect may be pro-inflammatory and immunosuppressing. Generally, immature cells at rest are stimulated by TGF-β, whereas if such cells are activated they may be inhibited by TGF-β. Therefore, it could be considered an anti-cytokine, that is, it provides negative regulation of immune response. Its inflammatory effects include macrophage chemotaxia and in lower scale fibroblasts, increase of adhesion molecule expression and TGF-β self-induction. It has an angiogenic effect promoting collagen and new vessel proliferation. Such findings suggest that excessive TGF-β production could result in scar formation, leading to the pathogenic mechanism of fibrosis [7]. Several authors have studied TGF-β in acquired cholesteatoma [8,19,20].

Transforming growth factor-Alpha (TGF-α) is a growth factor of polypeptides for epithelial and mesenchymal cells. TGF-α is produced by keratinocytes, macrophages, platelets, and its immune expression has the structure related to Epidermis Growth Factor (EGF) and binds itself to its receptor [7]. Several authors have studied TGF-α in acquired cholesteatoma [20-23].

Epidermis Growth Factor (EGF) is a polypeptide that stimulates epidermic cells, fibroblasts and endothelial cells (angiogenesis). Its receptor (EGF-R) is located in epithelial tissue[24]. Many authors have studied EGF and EGF-R in acquired cholesteatoma [13,21,25,26].

Keratinocyte Growth Factor (KGF) is a polypeptide that stimulates proliferation and differentiation of epidermic cells. It may be produced by fibroblasts. Its receptor (KGF-R) is located in the epithelial tissue[27]. Yamamoto-Fukuda et al. have studied KGF and KGF-R in acquired cholesteatoma [27].

DISCUSSION

Regarding the genesis of acquired cholesteatoma, many theories have been discussed. Currently, there is a consensus on its classification in primary and secondary and on the theories to explain the origin of each one of the subtypes. The pathogenesis of acquired cholesteatoma is directly related to middle ear affections, whether by dysfunction of the auditory tube or by infections originated in it, resulting in tympanic membrane perforation. Both the onset and evolution of cholesteatoma are multifactorial and related to genetic characteristics and molecular biology of its cells. The migratory potential of the canal and juxtatympanic cells is provided by cytokeratins with proliferative characteristics that could only be present in benign hyperproliferative diseases of the epidermis and in zones under pressure and friction. This potential is reactivated if stimulated by physical forces applying pressure or by an infectious process such as those occurring in cholesteatoma [4]. Once process starts, both inflammation resulting from secreting middle ear otitis and bacterial infection stimulate cytokine release. These properties provide a unique behavior to cholesteatoma, given that one can be more aggressive than the others. Some of such characteristics are recurrent and others not. [5]

Among the several structures found in cascade events of human body inflammatory response and homeostasia, cytokines have been the primary target in research studies. [7]

Innumerable cytokines have already been studied in acquired cholesteatoma and each one of them will be discussed in the present study.

Marenda & Aufdemorte[8] and Bujia et al. [10] described Interleukin 1 (IL1) in acquired cholesteatoma. They agreed in their findings reporting IL 1 immunoexpression in all layers of the epithelium and subepithelium of the cholesteatoma. Akimoto et al.[12] did not agree with those findings and reported IL-1 immunoexpression only in subepithelial tissue of cholesteatoma. This difference may be explained by the study conducted by Chung & Yoon[11], in which they performed a dissociation of epithelial from subepithelial tissue and used epithelial cells to perform tissue culture. These authors found out that epithelial cells produced IL-1 only under the influence of subepithelial tissue. This fact was described in immunology, since the main inducer of IL-1 synthesis is lymphocyte T, which is present in the subepithelium of the cholesteatoma.

In relation to the immune expression of interleukin 6 (IL-6) it can be said that there was a consensus among the authors. Both Marenda & Aufdemorte[8] and Bujia et al.[14] found it in all layers of the epithelial and subepithelial tissue of cholesteatoma.

Regarding the role of interleukins in cholesteatoma, IL-1 stimulates bone reabsorption increasing the number of osteoclast precursor cells, and also stimulates fibroblasts and osteoblasts production of prostaglandins and collagenases. IL-6 also induces osteoclast formation. High concentrations of IL-6 were related to erosion of ossicular chain and presence of intra-operative granulation tissue. IL-8 promotes the production of collagenases, which are proteins involved in process of bone lysis [8,10,11].

In reference to immune localization Tumor Necrosis Factor-Alpha (TNF-α) in acquired cholesteatoma, the authors disagreed. Yan & Huang[16] found TNF-α immune expression in basal and prickle cells of the epithelium and subepithelial tissue. Whereas Marenda & Aufdemorte[8] described TNF-α immune expression in suprabasal layers of the epithelium of human cholesteatoma and in subepithelial tissue, but it was not found in basal layers. Still in the study of Sastry et al.[18], TNF-α was immunoexpressed in all layers of the epithelium of cholesteatoma. Contrarily to those findings, Akimoto et al.[12] reported TNF-α immunoexpression only in subepithelial tissue of cholesteatoma.

With regard to TNF-α role in cholesteatoma, it acts as a mediator in tissue collapse and remodeling processes, increases the activity of collagenase, exposing bone surface to osteoclast action. It is an autocrine growth regulator stimulating protein synthesis, in which proliferation and differentiation of keratinocytes endings result in increased keratin production. Keratin has an inflammatory stimulation effect resulting in granular tissue formation. The TNF-α concentration is proportional to the level of bone destruction. TNF-α also has high plasma levels in patients with cholesteatoma, especially in those with massive bone destruction[18].

Authors had different opinion regarding TGF-β immunoexpression in cholesteatoma, as Lang et al.[19] and Sudhoff et al.[20] found it only in subepithelial tissue, whereas Marenda & Aufdemorte[8] found it in suprabasal layers of the epithelium and in subepithelial tissue of human cholesteatoma.

In reference to the role of TGF-β in cholesteatoma, it regulates osteoblast proliferation and differentiation, decreases the number of osteoclasts, stimulates macrophages and fibroblasts, in addition to inhibiting the action of proteolytic enzymes that destroy recently formed tissues, resulting in granulation tissue formation. TGF-$\beta2$ activity is related to the presence of bone erosion in middle ear and TGF-$\beta1$ is related to the evolution period of the disease. TGF-$\beta1$ inhibits osteoclast formation and other cytokines such as IL-1. Its presence probably decreases proliferation of cholesteatoma and keratin lamella formation. [8]

The authors had different opinion regarding TGF-α expression, since all of them found it in all layers of the epithelium of cholesteatoma [20-23,] and only some of them found TGF-α immunoexpression also in subepithelial tissue of cholesteatoma[20,22]. TGF-α interacts with EGFR present on the cell surface [23].

Epidermis Growth Factor (EGF) and its receptor (EGF-R) were studied in acquired cholesteatoma by many authors that agreed in their findings regarding the immunoexpression of EGF-R in all layers of the epithelium of cholesteatoma [21,25,26].

Keratinocyte Growth Factor (KGF) and its receptor (KGF-R) were studied in acquired cholesteatoma by Yamamoto-Fukuda et al.[27] that found KGF only in fibroblasts of subepithelial tissue, whereas KGF-R was found only in the epithelium of cholesteatoma. The study conducted by the authors showed some future perspective in the investigation of cholesteatoma, since the cytokine receptor is found in epithelial tissue and the cytokine itself is found in subepithelial tissue.

The inflammatory process that occurs in subepithelial tissue of cholesteatoma seems to play an important role in the production of cytokines that act both in subepithelial and epithelial tissues of cholesteatoma. This interaction between epithelium and subepithelium was demonstrated after tissue culture of cholesteatoma was performed, separating the epithelium from the subepithelium [11].

New studies should be conducted to compare differences among the pattern of cytokine expression between infected cholesteatoma and dry cholesteatoma, or still describe cytokine expression in the cover layer of epithelialized radical cavities to better understand this common disease in our field.

CLOSING REMARKS

The following cytokines, growth factors and receptors were reported in cholesteatoma: IL-1, IL-6, IL-8, TNF-α, TGF-α, TGF-β, EGF, EGF-R, KGF and KGF-R. The authors, however, had different opinions on the immune localization of each cytokine.

There is not only one cytokine responsible for each characteristic of the cholesteatoma, such as bone reabsorption, invasion and angiogenesis. In fact, there is synergism between different cytokines resulting in the aggressive characteristics of cholesteatoma.

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