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TNF-α及 IL-1β在人類中耳膽脂瘤之研究

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TNF-α and IL-1β in Human Middle Ear Cholesteatoma

中文摘要

關鍵詞: 膽脂瘤, 中耳炎, 發炎, 細胞素

中耳膽脂瘤係由類似皮膚的表皮細胞層及結締組織——包括肉芽組織及許多炎性細胞所組成，因表皮細胞不斷增生及分化的結果，使得膽脂瘤在中耳裂中愈來愈大。至於原來不存在有表皮細胞的中耳，其膽脂瘤表皮細胞的來源，以及什麼因素促成膽脂瘤的生長現象，都是研究者不斷在探討的專題。

今已知促成膽脂瘤的基底細胞不斷增生及分化的因素，與其併存的炎性細胞有密切關係。本研究中耳膽脂瘤併慢性中耳炎時，收集其分泌液，測定其中TNF-α及IL-1β之含量，並比較其在無膽脂瘤之慢性中耳炎之分泌液中之含量。結果顯示其在有膽脂瘤之慢性中耳炎之分泌液中之含量均較高，在慢性漿液性中耳炎之分泌液中之含量相對較低，此反應著其在膽脂瘤的發展，包括發炎性與臨床破壞性也許有一席角色。

英文摘要

Keywords: Cholesteatoma, Proliferation, Differentiation, Inflammation, Cytokins.

Cytokins are glycoproteins secreted from a wide variety of cells including leukocytes, endothelial cells, epithelial cells and fibroblasts. They are activated by antibody production, immune cell differentiation, cell proliferation and cell-specific chemotaxis. They play an important role in the process of immune response and inflammatory reactions.

This study detects the quantity of TNF-α and IL-1β in middle ear cholesteatoma either with active inflammatory discharge or without discharge condition. TNF-α levels are 39.6±11.06, 28.34±11.21, 32.8±12.23, and 13.34±3.22 in ears of group 1, group 2, group 3, and group 4 respectively. IL-1β levels are measured as 78.54±16.22, 45.32±18.21, 50.23±17.12, and 16.12±4.33 pg/mg TP respectively. They are higher in the discharge of COM with cholesteatoma and relatively lower in the effusion of OME. These events reflect their roles in the chronicity and inflammatory processes of the disease.

Introduction:
Middle ear cholesteatoma is a common middle ear disease, pathologically benign but clinically destructive in the temporal bone. Cholesteatoma is composed of epithelial matrix and connective tissue including granulation tissue and many inflammatory cells.

The pathogenesis of middle ear cholesteatoma has been investigated for a long years [1-8]. The presence of epithelial cells in the middle ear is the characteristics of cholesteatoma. Proliferation and differentiation of the epithelial (basal) cells are the crucial factors in growth of cholesteatoma clinically[1,3,9-11].

Our previous study also showed presence of PCNA and Ki-67 in cholesteatoma [16]. It was compatible with the hyperproliferative activity of cholesteatoma. The presence of EGF, TGF-alpha and EGF-R in the cholesteatoma tissue means that the growth of cholesteatoma is contributed by these factors.

A few studies have examined the proliferative activity and DNA content of cholesteatoma in the past [16,18]. Immunohistochemical staining of cholesteatomas localized PCNA, a marker of proliferative activity, in all 40 specimens in our previous study.

Our unpublished data also revealed diploid DNA content in cholesteatoma cells by flow cytometry [19]. This result deny the characteristic of neoplasm for cholesteatoma.[20-22]

As cytokins are glycoproteins secreted from a wide variety of cells, such as leukocytes, endothelial cells, epithelial cells and fibroblasts, they play an important role in the process of immune response and inflammatory reaction [23-27]. Middle ear cholesteatoma is always coexisting with active middle ear inflammation that shows foul otorrhea clinically. This study is to detect the concentration of TNF- and IL-1 in the discharge. It is hoped to have a further understanding of its development and destruction.

Materials & Methods

Aural discharge was collected with sterile collectors (Juhn TYM-TAP, Xomed -Treace) from 30 ears with cholesteatoma (group 1) and from 20 ears of chronic otitis media without cholesteatoma (group 2). Aural discharge also was obtained from 5 ears (group 3) with cholesteatoma without discharge by irrigation with 1.5ml normal saline. Another kind of middle ear fluid sample was collected from otitis media with effusion in 10 patients (group 4) with nasopharyngeal carcinoma after irradiation. The collected samples was first diluted by 1 ml PBS, and was centrifugated at 2000 rpm, -4℃. Supernatants was taken with micropipets and stored at deep freezer (-70℃) until use.

TNF- and IL-1 levels with be measured by ELISA method (ELISA kits; DAKO, Denmark). Sensitivity level of IL-1 is greater than 10 pg/ml and that of TNF- is greater than 15.6 pg/ml. Frozen samples were gradually warmed at room temperature prior to analysis, diluted and incubated with "mouse anti-human monoclonal antibody" and later with "goat anti-mouse conjugated alkaline phosphatase" and "color reagent A-B" (chromogenic substrate). Absorbent values was read at 420nm on microplate autoreader (EL-312 Microplate Biokinetics Reader). Standard values for TNF- and IL-1 and optic density of absorbents and concentrations in each sample were determined.
Results:

TNF-α and IL-β levels in the four group samples were obtained and analyzed. TNF-α levels are 39.6±11.06, 28.34±11.21, 32.8±12.23, and 13.34±3.22 in ears of group 1, group 2, group 3, and group 4 respectively. IL-1β levels are measured as 78.54±16.22, 45.32±18.21, 50.23±17.12, and 16.12±4.33 pg/mg TP respectively.

Discussion:

Cytokins are glycoproteins secreted from a wide variety of cells including leukocytes, endothelial cells, epithelial cells and fibroblasts. They are activated by antibody production, immune cell differentiation, cell proliferation and cell-specific chemotaxis.

Middle ear cholesteatoma is a common middle ear disease associated with chronic otitis media. It is composed of keratin, epithelial matrix and the subepithelial connective tissue containing granulation tissue and many inflammatory cells. Histologically, all the components are benign. Clinically, the cholesteatoma continuously grows and invades the surrounding structure in the temporal bone.

Clinically, cholesteatoma develops and expands basing on continuing proliferation and differentiation of its epithelial cells. As already known, there’s coexisting with granulation containing numerous inflammatory cells that provide some factors promoting the proliferation and differentiation of the basal cells of cholesteatoma. Regarding with the source of the epithelial cell which is absent in the normal middle ear and the factors promote its growth, they have been studying by many investigators. Recently, in studying the pathogenesis of cholesteatoma, in addition to the relationship among its composed cells, signal transduction pathway in epithelial cells related to the growth of cholesteatoma is getting more and more studies in the biological science. Our previous study revealed the expression of c-jun/c-fos, and p53 in the epithelia and subepithelial connective tissue. This suggests that the signal transduction cascade plays an important role in the growth and clinical development of cholesteatoma[12-15].

Inflammatory mediators in the middle ear which are released upon tissue injury have certain effects on transformation of acute otitis media into a chronic effusion [28]. Thus, identification of the behavior and the role of such mediators is important. There are increasing reports that great amount of cytokins are found in middle effusion. TNF has higher concentrations in the middle ear than IL and is responsible for chronicity of the disease [26]. It was found that IL is basically released from neutrophils and other leucocytes [29]. IL-1β and TNF-α cytokine levels in this study is measured to be higher in the discharge of COM with cholesteatoma than in the condition of COM. They are also lower in the condition of chronic OME and even in the condition of cholesteatoma without discharge.

The relation between the type of effusion and cytokine levels is quite interesting. IL-1β and TNF-α values in purulent discharge are higher than in serous and mucoid ones. Moreover, IL-1β levels in mucoid effusion are higher than in serous samples. These events reflect the course of the disease. An increase in viscosity of effusion presents a gradual and proportional correlation with the duration of the disease over time and cytokins play a role in the process [30].
Conclusion:

This study detected the quantity of TNF-α and IL-1 β are higher in middle ear cholesteatoma either with active inflammatory discharge or without discharge condition. They play an important role in the process of inflammatory reactions. TNF-α and IL-1 β have several biological properties and there are complex interactions between the clinical stage or other parameters and them. Further studies are needed to clarify their roles in the clinical behavior and it is hoped to provide a new concept for controlling the diseases.

References:
19. Chao WY. Flow cytometric analysis and proliferation activity study in cholesteatomas.[Unpublished data]