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OTOLOGY

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Cytokines in children with otitis media with effusion

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Abstract We assayed 38 middle ear effusions from 23 children aged 4–13 years (mean 7) undergoing tympanostomy tube placements. All fluid was assayed for tumor necrosis factor (TNF) α, interleukin (IL) 1β, IL-8, and IL-10. Cytokine concentrations were measured by means of an enzyme-linked immunosorbent assay. Detectable levels of IL-1β, IL-8, and IL-10 were found in all of the effusions. TNF-α was detected in 18 of the middle ear effusions (47.4%). The mean concentration of TNF-α, IL-1β, IL-8, and IL-10 was, respectively, 0.423 ± 1.39, 30.58 ± 68.7, 7001.9 ± 6743, and 56 ± 58.7 pg/ml. There was a strong, statistically significant correlation between the concentrations of TNF-α and IL-1β (r = 0.87, P = 0.001) and between IL-1β and IL-8 (r = 0.53, P = 0.001). There was no correlation between the concentrations of IL-10 and other cytokines examined or between tympanic membrane pathology and the concentrations of TNF-α, IL-1β, IL-8, or IL-10. The presence of IL-10 in middle ear effusions may be one of the causes of a lack of clinical features of acute inflammation and may lead to a chronic inflammatory state.

Keywords Otitis media with effusion · Cytokines · Enzyme-linked immunosorbent assay

Introduction

Otitis media with effusion (OME) is one of the major causes of hearing loss in children. This hearing loss may be responsible for delays in speech, language, and cognitive development. Chronic inflammation of the middle ear generates mucosal hypertrophy, adhesions, osteolysis, cartilage damage, deterioration of the fibrous layer of the tympanic membrane, and atelectasia. However, eustachian tube dysfunction and adenoid hypertrophy do not explain the pathogenesis of OME adequately.

Bacterial and/or viral infections, allergic reactions causing inflammatory changes in the middle ear and local immune dysfunction associated with the persistence of pathogenic bacteria or bacterial components have long been implicated in the etiology of OME. Many inflammatory mediators, such as the components of coagulation, fibrinolytic and complement systems, immunoglobulins, and immune complexes, have been identified in middle ear effusions (MEEs) and have been implicated as having an effect on the pathology of OME. Inflammatory cells and arachidonic acid derivatives have also been demonstrated in the middle ears of these patients [11].

Recently the role of cytokines, a group of glycoproteins which participate in the modulation of inflammatory and immune reactions in many diseases, has been emphasized in OME. Earlier studies have documented the presence of several cytokines, including tumor necrosis factor (TNF) α, interleukin (IL) 1β, IL-2, IL-6, and IL-8, interferon-γ, and TNF soluble receptor, in middle ear effusions in humans and experimental animals [3–6, 12, 19, 20]. In contrast, IL-4 and granulocyte-macrophage colony-stimulating factors have not been detected, lending support to the hypothesis that OME is mediated by specific cellular messages. IL-10 has been shown to inhibit formation of interferon-γ and production by macrophages of IL-1, IL-6, and TNF-α, but has not been investigated in middle ear effusions.

To our knowledge no previous study has examined the correlation between levels of cytokines in an effusion and destructive changes in the middle ear, such as atelectasia and tympanosclerosis of the tympanic membrane. The aim of this investigation was to measure the concentrations of TNF-α, IL-1β, IL-8, and IL-10 in MEEs, assess possible correlations and determine whether cytokine levels could be associated with clinical features.

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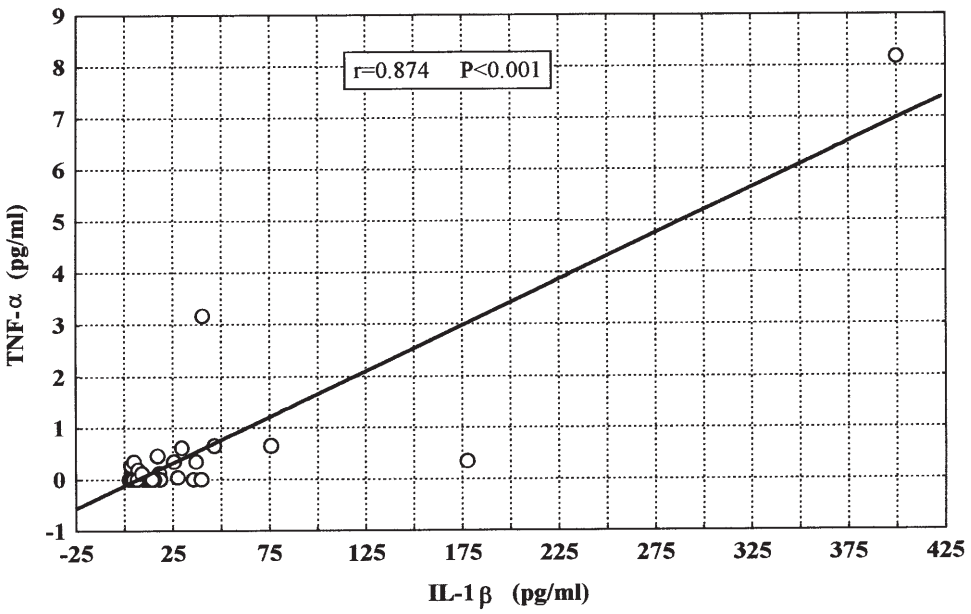

Fig. 1 Correlation between the concentration of TNF-α and IL-1β in children with MEEs (*r* linear Pearson's correlation coefficient)

Materials and methods

A total of 23 children (aged 4–13 years, mean 7; 15 boys, 8 girls) with serous otitis media were scheduled for myringotomies and placement of tympanostomy tubes. One 11-year-old girl had a myringotomy and adenotonsillectomy performed 4 years previously. In all other cases MEEs were collected at the time of their first tympanostomy tube placement. Other surgery carried out at the same time was adenoidectomy ($n = 20$), adenotonsillectomy ($n = 2$), and adenoidectomy with septoplasty ($n = 1$). Otoscopic examination in 10 cases (12 ears) showed retraction pockets. One child had tympanosclerotic plaques in the pars tensa of the tympanic membrane.

Tympanostomy tubes were placed under general anesthesia. MEEs were collected into Juhn Tym-Taps (Xomed-Treace, Jacksonville, Fla., USA) and classified by general appearance as mucoid or serous at the time of myringotomy. Specimens were immediately frozen at –80 °C for assays at a later date. MEEs were prepared for analysis by adding 1.3 ml phosphate-buffered saline (PBS) to each effusion and exposed to ultrasound for 10 s at a temperature of 4 °C. This was repeated three times. To every 10 µl homogenate 90 µl PBS was added. Total protein levels were determined in 20 µl diluted homogenate by the method described by Lovry et al. [10]. The rest of the diluted homogenate was used for cytokine concentration measurements.

Hearing losses in the children lasted from 4 months to 7 years (mean 1.17 years) before tympanostomy tube placement. The number of episodes of acute otitis media had ranged from 0 to 10 (mean 3.6). MEEs were collected from both ears in 15 cases and from one in 8 (5 right, 3 left). The fluid was serous in only three ears.

The diluted MEEs were assayed for cytokine levels using enzyme-linked immunosorbent assay (ELISA) kits incorporating monoclonal antibodies and ETI system reader (Sorin Biomedica, Bio-Tek, USA). Kits were used to assay for TNF-α, IL-1β, IL-8, IL-10 (Endogen, Cambridge, Mass., USA). Cytokine concentrations in the homogenate were determined by comparison to a standard curve plotted as absorbance versus the standard concentration. Homogenate for measuring IL-8 was diluted 20× in PBS. The level of cytokine was measured in probes and expressed as the mean (in picograms/milliliter). The sensitivity of these ELISA results was defined as the lowest concentrations of cytokine detectable in MEEs and was 5 pg/ml for TNF-α, 1 pg/ml for IL-1β, 2 pg/ml for IL-8, and 3 pg/ml for IL-10. The nonparametric Mann-Whitney *U* test was used for statistical analysis and Pearson's linear correlation coefficent was calculated. Statistical significance was defined at $P < 0.05$.

Results

The mean concentration of total protein was 18.62 mg/ml (5–46.5mg/ml). Using the ELISA system IL-1β, IL-8, and IL-10 were detected in all MEEs examined, while TNF-α was detected in 18 of 38 MEEs. The range of TNF-α levels was 0–8.17 pg/ml and the mean 0.423 ± 1.39. The range of IL-1β levels was 2.74–400 pg/ml and the mean 30.58 ± 68.7. The range of IL-8 levels was 238.6–20,000 pg/ml and the mean 7001.9 ± 6743. IL-10 levels varied between 3.3 and 289.9 pg/ml, and the mean was 56 58.7. There was a strong correlation between TNF-α and IL-1β concentrations (Fig. 1) and between IL-1β and IL-8 concentrations (Fig. 2). The correlation between TNF-α and IL-8 had limited statistical significance ($r = 0.31$; $P = 0.056$). However, no correlation was found between the concentrations of IL-10 and the other examined cytokines. There was no correlation between age, duration of hearing loss, number of episodes of acute otitis media, and the concentrations of the examined cytokines. Using the Mann-Whitney *U* test no statistically significant correlation was found between the tympanic membrane pathology during otoscopic examinations and the levels of cytokines.

Discussion

In the course of OME a number of inflammatory and immunological processes take place which act through a common cytokine system [11]. Previous studies have documented the presence of several cytokines in MEEs collected during tympanostomy tube placement and their significant role in the pathogenesis of OME. The proinflammatory cytokines TNF-α, IL-1β, IL-8, and IL-10 which inhibit their production are part of a local immunological response. TNF-α, IL-1β and IL-8 were present in a high percentage of the MEEs examined. In our study TNF-α

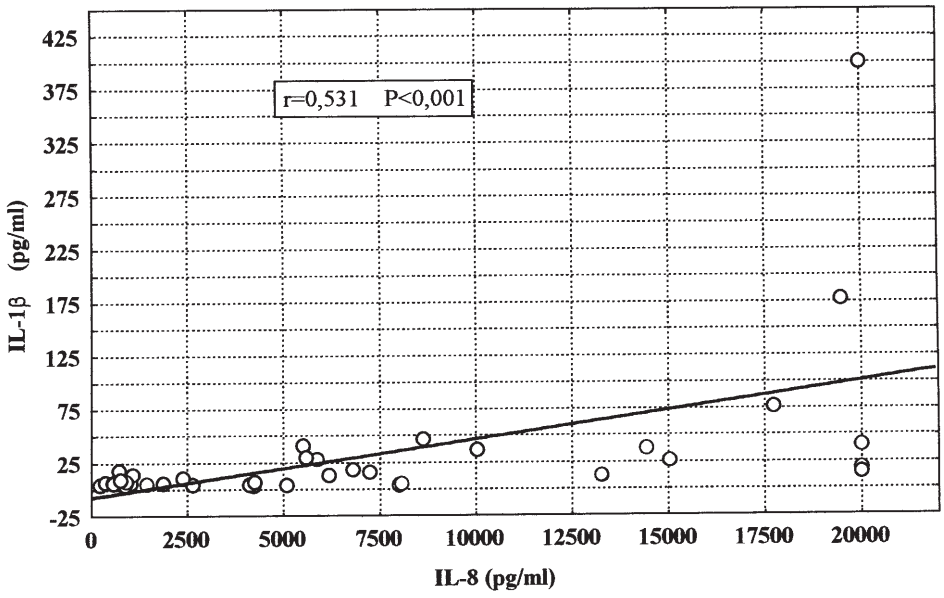

Fig. 2 Correlation between the concentration of IL-1β and IL-8 in children with MEEs (*r* linear Pearson's correlation coefficient)

was detected in 47.4% of specimens and IL-1β and IL-8 in 100%.

Various levels of TNF-α and IL-1β have been reported in MEEs [7, 11, 21]. Yellon et al. [21] and Ophir et al. [15] reported levels of TNF-α similar to our measurements. However, the values reported by Johnson et al. [5] are much higher, and there is no information in any of the papers concerning the duration of OME. In the group examined by us the mean duration of MEE was 1.2 years which could have an impact on our results. Endotoxin is a potent inducer of various inflammatory mediators and stimulates local macrophages to produce TNF-α [15]. However, endotoxin stimulates mainly the acute phase of the inflammatory response and acts as long as it is present in the middle ear. This can persist in the middle ear for up to 3 months [18], and the mean duration of illness in our group was much longer.

Previous studies have demonstrated IL-8 in a majority of MEEs [4, 6, 11, 14]. To our knowledge, ours is the first report documenting the presence of IL-10 in human MEE, and this was detected in all of our specimens. The mean conceration of IL-10 in our study was 56 pg/ml. According to Lacki et al. [8], the concentration of IL-10 in human serum is 7.2 pg/ml. Lehmann et al. [9] found that the concentration of IL-10 in the serum of patients with meningococcal shock was 21.2 pg/ml, while the mean level of IL-10 in bronchoalveolar lavage fluid from healthy humans was 130 pg/ml [1].

The presence of IL-10 in MEEs may be one of the causes of a lack of clinical features of acute inflammation and may lead to a hyporesponsiveness that could contribute to a chronic inflammatory state. Borish et al. [1] suggest that corticosteroids produce their anti-inflammatory effect by inducing the production of IL-10. IL-4 is another cytokine produced by Th2 lymphocytes and was not detected in MEEs. This may suggest that macrophages and not Th2 lymphocytes are the main source of IL-10 in MEEs.

We found a statistically significant correlation between the concentrations of TNF-α and IL-1β and between those of IL-1 and IL-8. This is in agreement with the observations by Maxwell et al. [11]. With respect to many inflammatory responses TNF-α and IL-1β are similar. They both activate neutrophils, induce the growth of fibroblasts, inhibit bone collagen synthesis, and allow osteoclastic resorption. TNF-α and IL-1β are strong inducers of IL-8 production [11, 15, 19].

The presence of IL-8 in all of our MEEs examined and the correlation of their concentration with levels of IL-1β further supports the hypothesis of Maxwell et al. [11] that IL-8 expression is under the direct control of inducer cytokines IL-1β and TNF-α, and IL-8 is crucial in the leukocyte response in the middle ear and pivotal in the maintenance of inflammation in OME.

We found no correlation between the concentrations of IL-10 and the other cytokines examined. IL-10 is produced by activated Th2 lymphocytes and its main role is the suppression of Th1 cells. It can also be produced by monocytes and has an autoregulatory role in the inhibition of cytokine synthesis by activated human monocytes [17]. IL-10 has been implicated in the selective enhancement of IgA production [2], which is consistent with the increased local production of IgA during chronic OME [13]. In other studies IL-10 has been shown to inhibit the production of TNF-α and IL-1β in tissue cultures of colon in a murine model of colitis [16].

We found no correlation between age, duration of hearing loss, number of episodes of acute otitis media, and the concentrations of the cytokines examined. Opinions about correlations between the clinical features and the cytokine levels vary among authors. Maxwell et al. [11] and Yellon et al. [19] found a positive correlation between age and TNF-α concentration as opposed to IL-1β which has been associated with a younger age [11, 19, 20]. However, Yellon et al. [21] in their later study did not confirm their pre-

vious correlation between age and IL-1β. Hotomi et al. [4] demonstrated that the mean level of IL-8 in MEEs from children was higher than that from adults. Additionally, IL-8 concentrations have been found to be correlated positively with the type of effusion and the total number of neutrophils in MEEs [4, 14].

According to Yellon et al. [19], children undergoing repeat tympanostomies had mean TNF-α levels that were nearly 14 times higher than those from children undergoing their first tympanostomy. The same correlation was shown found in relation to IL-1β and IL-6 [21]. In contrast, Maxwell et al. [11] found that the highest levels of IL-1β were associated with children who had not had previous tube insertions but were using medications, and IL-8 and TNF-α levels did not differ significantly in terms of the number of previous tube insertions. However, the same authors later stated that TNF-α showed a significant trend for lower values as the number of tube insertions increased, and that children who subsequently developed at least one episode of otitis media after tube placement were much more likely to have higher levels of TNF-α in their effusions [12]. Nonetheless, Willett et al. [18] did not find significant correlation between the number of previous tube insertions and the concentrations of TNF-α and IL-1β in MEEs.

The lack of a significant correlation between clinical features and cytokines in MEEs in our study may in part reflect the differences in the number of patients examined and the types of patients enrolled. Our patient population was limited to children with chronic or persistent OME, and our patients were older than the children examined by Yellon et al. [20, 21] and Maxwell et al. [11, 12]. The significance of this remains to be confirmed in future studies.

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