Inflammatory Mediators in the Pathogenesis of Otitis Media: A Brief Review

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Otitis media (OM) is a common childhood disease. OM can be defined as an inflammation or infection of the middle ear cavity. Among many causative factors of OM, infection and eustachian tube (ET) dysfunction are most important. These causative factors stimulate middle ear epithelia and inflammatory cells to secrete inflammatory mediators (IMs) such as cytokines, eicosanoids, platelet-activating factor (PAF), tumor necrosis factor (TNF)-a, and nitric oxide. These IMs increase vascular permeability and secretory activity resulting in middle ear effusion (MEE) [1]. Thus, IMs play a central role in the pathogenesis of OM.

Pathogenesis of OM has been studied in humans and various animal models. Various forms of OM have been studied in longitudinal and parallel studies including acute purulent otitis media (POM), serous otitis media (SOM), mucoid or secretory otitis media (MOM), and chronic suppurative otitis media (COM). When these forms of OMs are followed longitudinally, they seem to change in continuum and interrelated [2].

The purpose of this study was to better understand the role of IMs in the pathogenesis of OM by finding levels of IMs in samples of human middle ear effusion (MEE) and in animal models of OMs and by studying the effect of the therapeutic use of inhibitors of IMs.

Methods

The topic of IMs in the pathogenesis of OM was searched in the National Center for Biotechnology Information (NCBI) and at the U.S. National Library of Medicine (NLM).The articles were reviewed, organized, and discussed. Only common IMs studied in OM were included. Most of the studies start with assaying samples of MEE in humans obtained at the time of myringotomy and tympanostomy tube (TT) insertions or in different types of animal models at different time intervals. Many of the animal studies also examined corresponding temporal bone histopathology.

Results

Lysozyme and Lactic Dehydrogenase (LDH)

Both lysozyme and LDH have been used as an index of inflammation.

Human MEE

Levels of lysozyme and LDH were higher in mucoid MEE than serous MEE [2].

Animal MEE

In POM model using chinchilla infected with pneumococcus, concentration of lysozyme and LDH were highest on day 7 and decrease over time. In SOM model induced by blocking eustachian tube in chinchilla and cat, levels of these IMs were lower than POM [2].

Histopathology of the temporal bones showed inflammations in mucoperiosteum corresponding to levels of IMs [2].

Eicosanoids, Prostaglandins (PGs), and Leukotrienes (LTs)

Among the various IMs of OM, arachidonic acid (AA) metabolites (eicosanoids) such as PGs and LTs appear to play an important role in the pathogenesis of OM. To investigate the role of AA metabolites on the pathogenesis of OM, concentrations of AA metabolites were measured in the MEE from human and paralleling animal models of OM and effects of inhibiters of AA metabolism, antibiotics, and tympanostomy tube (TT) on the outcome of animal models of

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OM were studied [3]. AA is released from membrane phospholipids by the action of phospholipase. This step is inhibited by corticosteroids. Once released, AA can be converted to two metabolic pathways, one through cyclo-oxygenase into PGs and the other through lipoxygenase into LTs. Most of nonsteroidal anti-inflammatory drugs (NSAIDS) like aspirin or ibuprofen inhibit the activity of cyclo-oxygenase.

Human and Animal MEE

PGE2 and PGF2a in human pooled MEE were measured by radioimmunoassay (RIA) and found that mucoid effusions had higher concentrations of PGs than serous effusion [4]. In more extensive studies concentrations of major AA metabolites, both PGs and LTs were assayed and analyzed according to type of fluids, age groups, and bacterial culture results. It was found that AA metabolites were higher in younger age group than from older age groups. Levels of LTs were generally higher in mucoid MEEs than in serous MEEs. LTB4 was the only AA metabolite with higher levels in bacterial culture-positive human MEE.

Effect of Inhibitors of AA Metabolism

From the study of the therapeutic use of inhibitors of AA metabolism, combination of penicillin and corticosteroid was the best mode of therapy, followed by penicillin alone and penicillin plus NSAID. Therapy with ibuprofen alone induces more inflammation and increased effusion probably because of increased LTs due to inhibition of cyclo-oxygenase [3].

Effect of LT and Its Inhibitor on the Clearance of the Eustachian Tube

Using guinea pigs, this study demonstrated that LTC4 impaired mucociliary clearance function of the eustachian tube in dose-dependent manner. This inhibition of mucociliary clearance function was prevented by pretreatment with LT inhibitor. The findings of this study suggest that LT plays an important role in the pathogenesis of OME by impairing eustachian tube clearance function. LT inhibitors may have future prophylactic or therapeutic implications for OME [5].

Platelet-Activating Factor (PAF)

A biologically active phospholipid PAF is known to have wide-ranging effects on acute inflammation and allergy. It is released from human neutrophils, platelets, eosinophils, macrophages, mast cells, and vascular endothelial cells [6]. It has been identified in human MEE and has been suggested to participate in developing OME and maintaining MEE by stimulating vascular permeability and chemotaxis [7].

To test hypothesis that PAF can induce OME, PAF was injected into the chinchilla bullae which induced dose-dependent OME. PAF antagonist, WEB 2170, prevented the development of OME. The findings of this study suggest that PAF plays an important role in the pathogenesis of OME [8].

Tumor Necrosis Factor-a (TNF-a)

TNF is produced by activated macrophages and has various activities. It activates neutrophils, induces production of intercellular adhesion molecules from lymphocyte, directs tumoricidal action on certain tumor cell line, enhances production of interleukins such as interferon, interleukin (IL)-1, IL-2, IL-6, and IL-8, and stimulates production of collagenase, prostaglandin, and pyrogen [9].

To clarify the role of TNF in experimental OME, OME was induced by transtympanic injection of TNF in rats. MEE was developed in 70% of specimens, and histopathological changes such as subepithelial edema and marked infiltration of neutrophils were present in 100% at 24 h after injection. MEE was prevented by pretreatment with TNF antagonist, TNF-soluble receptor type I (TNFsolRI) [10]. Another study performed by same group showed that treatment with TNF antagonist prevents MEE induced by pseudomonas lipopolysaccharide (LPS) in rats[11].

Nitric Oxide Metabolites

Free radicals such as nitric oxide (NO) seem to be important in the pathogenesis of OME. This study measured concentrations of NO in human MEE. Type of MEE was determined at the time of collection as SOM, MOM, and POM. Concentrations of NO metabolites were highest in MOM followed by SOM and POM. This study suggests that NO is present in human MEE and may play an important role in the pathogenesis of OME [12].

Review of IMS, OM, and Connection to Inner Ear Function

Here is a review article dealing with the characteristics of various inflammatory mediators identified in the middle ear during otitis media and in cholesteatoma. The role of each inflammatory mediator in the pathogenesis of otitis media and cholesteatoma has been discussed. Further, the relation of each inflammatory mediator to the pathophysiology of the middle and inner ear along with its mechanisms of histopathological change has been described. The mechanisms of hearing loss including sensorineural hearing loss (SNHL) as a sequela of otitis media are also discussed. The passage of inflammatory mediators through the round window membrane into the scala tympani is discussed. In an experimental

animal model, an application of cytokines and lipopolysaccharide (LPS), a bacterial toxin, on the round window membrane induced sensorineural hearing loss as identified through auditory brainstem response threshold shifts. An increase in permeability of the blood–labyrinth barrier (BLB) was observed following the application of these inflammatory mediators and LPS[13].

Inflammasome and OME

The nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome is a critical molecule mediating interleukin-1β (IL-1β) responses. However, the role of the NLRP3 inflammasome in otitis media has not been fully examined. The purpose of this study was to assess the expression of NLRP3, ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain and a pyrin domain), and caspase-1 in lipopolysaccharide-induced otitis media. BALB/c mice received a transtympanic injection of either lipopolysaccharide or phosphate-buffered saline. The mice were sacrificed 24 h after injection. Concentrations of IL-1β, NLRP3, ASC, and caspase-1 in the middle ear effusions were measured by enzyme-linked immunosorbent assay. Temporal bones were processed for histologic examination and immunohistochemistry.

The transtympanic injection of lipopolysaccharide significantly upregulated levels of IL-1β, NLRP3, ASC, and caspase-1 in the middle ear as compared with the control mice. The proteins of NLRP3, ASC, and caspase-1 were observed in infiltrating inflammatory cells induced by lipopolysaccharide in the middle ear cavity. Lipopolysaccharide induces NLRP3 inflammasome components in the middle ear. The NLRP3 inflammasome may play an important role in the pathogenesis of otitis media. Modulation of inflammasome-mediated inflammation may be a novel therapeutic strategy for otitis media [14].

Viral and Bacterial Pathogens and IMs in MEE

Secretory *otitis media* (SOM) is characterized by persistence of fluid in the middle ear, often following an episode of acute *otitis media*. The hypothesis is that failure to eliminate bacterial or viral pathogens may result in persistent low-grade inflammation. In this study, inflammatory mediators in middle ear fluids from 67 children with SOM were analyzed. This was combined with determinations of viable bacteria by culture along with detection of bacterial and viral genetic material by real-time polymerase chain reaction (PCR). The inflammatory mediators found at the highest concentrations (>30 ng/mL) were stem cell growth factor-β (median 110 ng/mL), CXCL1, IL-16, IL-8, migration inhibitory factor, CXCL10, and CXCL9. Among bacterial pathogens, *Moraxella catarrhalis* and *Haemophilus influenzae* dominated, regardless of detection methods, while rhinovirus dominated among viral pathogens. Middle ear fluid levels of interleukin (IL)-1α, IL-17, IL-1β, fibroblast growth factor basic, and tumor necrosis factor correlated strongly with the presence of bacteria detected either by culture or PCR, while IL-1RA, IL-3, IL-6, IL-8, CCL3, CCL4, and granulocyte colony-stimulating factor correlated significantly with real-time PCR values. CXCL10, CXCL9, CCL2, and TRAIL correlated significantly with viral nucleic acid levels. To conclude, persistence of viral and bacterial pathogens may fuel persistent inflammation in SOM. Bacteria caused a broad inflammatory response, while viruses chiefly elicited the interferon-induced chemokines CXCL9 and CXCL10 [15].

Discussion

The goal of this review is to find the role of IMs on the pathogenesis of OM. Typical OM starts with viral upper respiratory infection followed by bacterial infection. It may start with eustachian tube (ET) dysfunction creating negative pressure in the middle ear. Frequently, OM may start with the combination of both bacterial infection and ET dysfunction. Either bacterial infection or ET or both stimulate middle ear epithelia and inflammatory cells to secrete inflammatory mediators (IMs) such as cytokines, eicosanoids, platelet-activating factor (PAF), tumor necrosis factor (TNF)-a, and nitric oxide. These IMs increase vascular permeability and secretory activity resulting in middle ear effusion (MEE) [1]. IMs can start, perpetuate MEE, and cause sequelae such as sensorineural hearing loss.

Most of the studies of IMs in OM start with assaying samples of MEE from human or animal model of OM. Usually, samples are divided by the type of fluids such as SOM, MOM, or POM either from human or experimental animal models.

Next step is injecting IMs either transtympanic membrane or through the top of the bullae and checking whether MEE is produced or not. This step is followed by treating with blockers of IMs being tested and checking whether formation of MEE is blocked or not. As much as possible histopathology of middle ear mucosa is examined.

The IMs that have been gone through these tests include cytokines, prostaglandins, leukotrienes, platelet-activating factor (PAF), tumor necrosis factor (TNF)-a, and nitric oxide. No doubt there are more IMs involved in the pathogenesis of OM, and more will be discovered and added to the list.

Goal of these studies is to find better treatment of OM. Finding how IMs induce OMs and inhibitors of IMs may reduce or block OM can be used clinically to treat

patients with OM. One example of this is the judicious use of glucocorticoid with antibiotics to treat POM. This practice came from extensive animal and human studies using glucocorticoid which blocks the formation of both LTs and PGs from AA. It will be possible that more inhibitors of IMs could be used to treat OM in the future.

Conclusion

This brief review demonstrates that various IMs such as lysozyme, LDH, PGs, LTs, PAF, TNF, ILs, and NO are involved in the pathogenesis of OM. These IMs were measured in the samples of MEE in human and animal models of OME and histopathological effect studied. Effects of blockers of these IMs on the outcome of animal models of OM were studied to discover better treatment for OM. One example of such finding was better outcome when OM was treated with antibiotic in combination with glucocorticoids.

Goal of these studies is to find therapeutic use of inhibitors of IMs. Further study is needed to determine if more of these IM blockers can be implemented into the clinical setting.

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