

Cytokine Responses in the Common Cold and Otitis Media

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Abstract Cytokines are a group of diverse molecules that influence the function of every organ system. They are most well studied in their effects on the immune system and their integral role in mediating inflammation. The common cold and otitis media are two such disease states, and much has been learned about the various effects of cytokines in each disease. Most often the viruses isolated include rhinovirus (RV), respiratory syncytial virus (RSV), adenovirus, coronavirus, and picornavirus. Otitis media, sinusitis, bronchiolitis, pneumonia, and asthma exacerbation are commonly accepted as complications of viral upper respiratory tract infections. Furthermore, otitis media and upper respiratory infections are inextricably linked in that the majority (>70 %) of cases of acute otitis media occur as complications of the common cold. Cytokine polymorphisms have been associated with the severity of colds as well as the frequency of otitis media. This article attempts to update the reader on various studies that have recently been published regarding the role of cytokines in these two disease entities.

Keywords Cytokine · Cytokine response · Otitis media · Common cold · Viral upper respiratory infection · Stress · Viral signaling pathways · Inflammatory response · Inner ear inflammation · Ion transport

Introduction

The common cold is a viral upper respiratory infection (vURI) that is caused by a number of different viruses such as rhinovirus, adenovirus, coronavirus, enterovirus, influenza virus,

parainfluenza virus, and respiratory syncytial virus (RSV) [1, 2]. Within the last decade, human bocavirus and human metapneumonovirus have been discovered and identified in middle ear effusions [3–5]. The degree of illness seen with an individual vURTI differs substantially, and a certain proportion of vURI can exist without causing a cold-like illness (CLI). Furthermore, complications of vURI (otitis media, sinusitis, acute bronchiolitis, pneumonia, and exacerbations of various respiratory illnesses including asthma and chronic obstructive pulmonary disease) can occur without CLI [6]. Thus, discussing the common cold can be done by analyzing studies of vURIs.

vURIs are a leading cause of health-care utilization. According to 2000 US census data, approximately 110 million physician visits, 23.2 million physician phone calls, and 6 million emergency department visits were attributable to vURIs [7]. Excluding asthma exacerbations, it was estimated that vURIs resulted in health-care expenditures approximating 40 billion dollars [7].

Otitis media can be an acute or chronic inflammatory condition involving the middle ear and mastoid. Otitis media is multifactorial with many variables affecting its outcome including environmental exposures, genetic characteristics, and infections. Specifically, acute otitis media occurs at least once in 80 % of children under the age of 3 and is the most common cause of antibiotic prescription in children [8, 9]. Otitis media is considered a complication of vURI, and otitis media is associated with preceding vURI in more than 70 % of cases [6].

Cytokines consist of a diverse group of molecules that allow intercellular communication. Cytokines are central to the inflammatory response and tissue remodeling. They have effects on a wide variety of organ systems and are implicated in numerous disease processes such as vURIs and the common cold. In this review, we explore the recent literature regarding cytokine responses secondary to the common cold and otitis media and their interactions.

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Cytokine Responses and the Common Cold

Cytokines' role in vURI is significant and appears to have importance in the variability of disease symptomatology. This is supported by greater nasal cytokine concentrations during vURIs versus disease-free periods [10–12], vURIs with CLIs versus vURIs without CLIs [13], and vURIs with airway complications [14–16]. Additionally, cytokine polymorphisms can be associated with increased susceptibility to vURTI, as seen with IL-6⁻¹⁷⁴ [17]. Disease severity also appears to correlate with cytokine polymorphisms, as seen with respiratory syncytial virus infections in 77 children [18]. Recently, Broers et al. supported this by reporting on an ex vivo study comparing cytokine response to influenza A virus exposure in children with trisomy 21 and in their siblings [19]. They found that TNF- α , IL-1 β , IL-6, and IL-8 were significantly higher in children with Down syndrome versus controls. Furthermore, they identified that baseline and post-viral levels of IFN- α and IFN- γ were significantly elevated. This suggests that in addition to having neurological impairment, cardiopulmonary defects, and abnormal upper airway anatomy, the innate cytokine response likely has an important role in increased disease severity with viral respiratory illness in Down syndrome patients and may even correlate with complications of vURIs, such as otitis media.

Cytokine Production in the Common Cold and Stress

It is commonly accepted that psychological stress increases the risk of many medical problems, including upper respiratory infections, poor wound healing, diabetes, and autoimmune and cardiovascular diseases [20]. Historically, this association has been attributed to dysregulation of the hypothalamic-pituitary-adrenocortical axis. Cohen et al. recently reported their unique study assessing chronic stress, glucocorticoid receptor resistance, inflammation, and risk of developing a cold when subjects were exposed to rhinovirus [21]. They showed that circulating levels of cortisol did not predict risk of cold, but that glucocorticoid resistance predicts an increased risk of cold. Perhaps more importantly, glucocorticoid receptor resistance correlated with increased IL-6 and TNF- α , but not with IL-1 β . Overall, this study supports the notion that induction of proinflammatory cytokines does not upregulate immunity, but may actually correlate to increased risk of infection.

Viral Signaling Pathways and Effect on Cytokines

Viruses infect host cells and depend on certain molecular processes present in host cells that allow replication. Host cells recognize this invasion, and protective mechanisms,

both innate and learned, exist to fight these organisms. During influenza infection, activation of NF kappa B and the Raf/MEK/ERK cascade is critical to viral replication. Specifically, inhibition of the Raf/MEK/ERK cascade and NF kappa B impaired both viral replication and the production of IL-8, MCP-1, IL-6, RANTES, IFN- β , and TNF- α in recent *in vitro* and *in vivo* models [22]. The idea that an inhibitor can be used to affect viral replication and cytokine production is an ideal therapeutic model. Such therapy, particularly if topical, could have obvious implications for the treatment of vURI and the prevention of complications such as acute otitis media.

Viral modification of Inflammatory Response Increasing its Virulence

Specific viral infections have been shown to increase the chance and virulence of bacterial infections [23]. Whereas the biology is not completely understood, viral and bacterial synergism has been theorized to be secondary to viral damage to host cells, local immunomodulation, and changes in the local environment that render the host fertile for bacterial infection [20]. Synergism has been identified in vURI and otitis media. Respiratory syncytial virus is a well-known cause of respiratory tract infections and has been strongly associated with acute otitis media [24–26]. A high RSV viral load is associated with an increased odds ratio of *Streptococcus pneumoniae* and *Haemophilus influenzae* acute otitis media. Recently, data have implicated special mechanisms that allow RSV induction of proinflammatory cytokines that cause immune modulation and downregulation of IFN- γ [27]. It is theoretical, but quite possible, that immune modulation and dysfunction such as this determine the risk of developing otitis media.

Cytokine Response, Upper Respiratory Infection, and Otitis Media

Cytokine response in vURTI has also been correlated to the development of acute otitis media [28]. Patel et al. [25] determined that an elevated nasopharyngeal IL-1 β concentration correlated with an increased risk of acute otitis media. Correlating this data with that of the Down syndrome study, it is possible that the predisposition to otitis media seen in Down syndrome is related to the cytokine response in addition to Eustachian tube dysfunction. If cytokines were associated with the risk of otitis media, one would think that a reduction in cytokine response could achieve decreased rates of otitis. Heinonen et al. found that treatment (oseltamivir) of influenza A within 24 h of onset significantly reduced the time to resolution from 6.5 to 3 days [29].

Interestingly, treatment within 12 h, but not 24 h, correlated with an 85 % decreased incidence of acute otitis media. How this drug resulted in a decreased rate of acute otitis media is not known, but one could make an argument that reduction of the initial proinflammatory cytokines is central to this biologic effect.

Cytokines and Otitis Media

Much time and effort has been spent evaluating the role of cytokines in otitis media throughout the years [30]. Cytokines can be released from numerous cell types found within the middle ear cleft including epithelial, endothelial, and immune cells of all types. Over the past year and a half, a few studies were published that increased our collective knowledge of the various cytokines present in otitis media. Other studies have helped to characterize the cytokines involved in specific types of conditions that may contribute to otitis media.

Tumor necrosis factor (TNFA, formerly known as TNF α) and its role in otitis media have been well documented [31]. TNFA causes the release of other inflammatory cytokines and is one of the most potent inducers of inflammation in otitis media. It is often found in high concentrations of middle ear effusions and is capable of creating otitis media with effusion independent of an infectious state [32, 33]. Moreover, TNFA expressed in higher concentrations is associated with an increased frequency and duration of otitis media [34, 35]. Despite these negative effects of TNFA, its absence (TNFA deficient mice) is associated with prolonged duration and inability to eradicate bacteria from the middle ear [36]. Coupling this fact with the fact that TNFA has the profound ability to induce apoptosis [37, 38], Ebmeyer et al. sought to identify the role of TNF-mediated apoptosis during otitis media [39]. They used a mouse model and compared wild-type to TNFA deficient mice after inducing experimental otitis media using nontypeable *H. influenzae*. They showed that epithelial and stromal thickness was significantly greater in the untreated state and after day 5 when the wild-type mice started to show recovery of middle ear cells. Furthermore, the TNFA knockouts developed polyps in a much higher proportion compared to wild-type mice. Lastly, the rate of apoptosis was assessed, and there was a delay of 2 days to peak apoptosis in the TNFA-deficient mice. Overall, this study indicates that TNFA is critical for cell turnover in healthy and diseased states, in addition to its widely known inflammatory effects.

Vascular endothelial growth factor (VEGF) increases vascular permeability greatly ($5 \times 10^4 \times$ histamine) [40]. VEGF production is stimulated by hypoxia, endotoxin, and TNF- α [41]. Recombinant VEGF (rVEGF) experimentally produces middle ear effusion, cellular infiltration,

subepithelial edema, and vascular dilation [42]. With this in mind, Sekiyama et al. analyzed the middle ear effusions of 33 children for the presence of VEGF, IL-8, endotoxin concentration, and albumin levels [43]. This study determined that VEGF, albumin, IL-8, and endotoxin were present in 100 %, 100 %, 98 %, and 89 %, respectively, of the 46 effusions. Comparing mucoid versus serous effusions, VEGF, IL-8, and endotoxin concentrations were significantly greater. Analyzing the concentrations within each class of effusion found VEGF to be correlated to endotoxin and albumin only within the mucoid effusions. Albumin is not produced in the middle ear and VEGF concentrations are much lower in the serum, allowing the correlation in mucoid effusions to be a rational conclusion. The fact that they are not correlated in serous effusions seems to be contradictory; however, other mediators of vascular permeability, such as histamine, platelet-activating factor, and other inflammatory products, must be considered. Overall, this study suggests that endotoxins can directly stimulate VEGF production and subsequently mucoid middle ear effusion.

Cytokine Response in Inner Ear Inflammation Associated with Otitis Media

As can be expected, much research has been performed on the cytokine response in otitis media and specifically the response in the middle ear. With otitis media, the inner ear is also subject to inflammation. As stated earlier, chronic otitis media can cause tissue remodeling and deleterious consequences including sensorineural hearing loss, speech delay, and learning difficulties. This tissue remodeling also occurs in the inner ear, causing decreased inner ear hair cells, spiral ganglion cells, accumulation of perilymphatic inflammatory cells, and scarring of the stria vascularis and spiral ligament [44]. These inner ear processes are caused by diffusion of cytokines through the round window and by intracochlear inflammatory responses to middle ear pathogens [45, 46]. To further our understanding of the inner ear inflammatory response, MacArthur et al. investigated middle ear and cochlear cytokine gene expression in both AOM and COM using separate mouse models for each [47]. This investigation included analysis of both inflammatory and tissue remodeling cytokines. The acute otitis media model resulted in significant middle ear expression of IL-1 α , IL-1 β , IL-6, and less potent production of TNF- α and BMP7. The acute otitis media model resulted in significant inner ear expression of IL-1 α , IL-1 β , IL-6, TNF- α , TGF β_3 , and VEGF α .

In the chronic otitis media model, the expression of middle ear cytokines was significant for an 18-fold increase in IL-1 α , 44-fold increase in IL-1 β , and 4-fold increases in TNF- α and VEGF α , while the remodeling cytokines of BMP6, BMP7, FGF1, and FGF3 were decreased. The

expression of inner ear cytokines showed more upregulation affecting IL-1 α , IL-1 β , IL-2, IL-6, TNF- α , TGF β ₃, and VEGF α , as well as BMP7 and FGF1. The cytokine expression in the chronic condition was significantly elevated compared to the acute otitis media in both middle and inner ear profiles. This study is significant as it provides evidence (and quantifies it) that direct gene expression occurs in cochlear tissue secondary to otitis media for the first time [44]. It is also important that this occurs in a number of inflammatory and tissue-remodeling cytokines, which can have obvious implications in end organ damage resulting in sensorineural hearing loss. What this study does not reveal is the mechanism of how cochlear tissues communicate with the middle ear. Does cochlear gene expression occur because of the bacterial components, or is it due to inflammatory molecules crossing the round window, or both?

Cytokines and Ion Transport

Throughout living organisms, ion and water transport is essential for proper function. Ion and water transport is vital to middle ear homeostasis, as shown in studies suggesting active roles for epithelial sodium channels and aquaporins [48, 49]. Additionally, IL-1 β suppresses the epithelial sodium channel and sodium channel-dependent fluid absorption in middle ear cells [50]. MacArthur et al. recently published their work determining the relationship between inflammatory gene expression in acute otitis media and various ion homeostasis genes within the middle ear [51••]. Their experimental model demonstrated significant upregulation of inflammatory genes and concomitant downregulation of most ion homeostasis genes studied, including aquaporins 1, 2, and 5, Na⁺K⁺ATPases, claudins (Cldn3^a), gap junction beta-6 (GJB6), epithelial sodium channel (ENaC), and K⁺ ion transport channel [48]. This suggests that downregulation of many ion transport genes may be critical to understanding the pathophysiology of middle ear fluid accumulation during otitis media. Further studies would be helpful to determine how and when upregulation of ion homeostasis genes occurs with relationship to the decreasing inflammatory response. Future therapeutic options may exist, and whether therapeutic upregulation of ion homeostatic genes can be created as a treatment of chronic otitis media needs to be determined.

Cytokine Response to Bacteria and Bacterial Products in Otitis Media

Middle ear inflammation and cytokine expression in middle ear effusion are often correlated with bacterial infection, while others support a viral etiology for the release of

cytokines. Recently, Stol et al. added to this body of literature by evaluating middle ear fluid in 116 patients (ages 5 and under) undergoing tympanostomy tube insertion [52]. They used quantitative PCR techniques to isolate various bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*) and 15 respiratory viruses from middle ear fluid. Additionally, they determined the concentrations of IL-1 β , IL-6, IL-8, IL-10, IL-17a, and TNF- α . The patients were grouped by presence of bacteria (27 %), virus (28 %), both (27 %), or neither (19 %) in the middle ear fluid. A significantly higher concentration of IL-1 β , IL-6, IL-8, and IL-10 was identified in middle ears containing bacteria, virus, and neither virus nor bacteria. Recurrent and chronic otitis media levels of cytokines were similar as well. The concentrations of various cytokines were tabulated against the PCR results. Within the middle ears that were positive for bacteria, *H. influenzae* had significantly more cytokines than *M. catarrhalis* and *S. pneumoniae*.

Overall, these results suggest the presence of bacteria or bacterial components is associated with a greater concentration of cytokines than viruses in subjects who are not actively infected. This may have significance in chronic otitis media; however, this study has limitations. The study did not evaluate the duration of the effusion or duration since the last acute infection. The presence of bacteria could represent a more recent acute infection, which would be associated with higher levels of cytokines that are in the process of declining. Thus, it is just as likely that the significant difference seen in this study was associated with the duration since the last acute infection.

The importance of bacteria and the inflammatory response they incite was evaluated in another recent study by Lee et al. [53]. This study identified cytokines from the transforming growth factor (TGF) family as having a significant role in tissue remodeling. The various TGF isomers are highly regulated and are critical actors in the formation of fibrosis and granulation tissue [54]. This study evaluated the TGF- β signaling pathway occurring in a rat model of acute otitis media by inoculation with *S. pneumococcus* (Pn6A), nontypable *H. influenzae* (NTHi), or eustachian tube obstruction (ETO). Pn6A and NTHi, to a much larger extent, increased the TGF pathway activity, whereas controls and ETO did not. Not insignificantly, the increased activity induced by NTHi was associated with an increased proliferation of the middle ear submucosal layer compared to both control and Pn6A. ETO produced results similar to those in controls, suggesting that eustachian tube dysfunction does not cause the proliferation of granulation tissue or subsequent fibrosis without bacterial infection.

Cytokine Production Due to Lipopolysaccharide (LPS)-Induced Otitis Media

Lipopolysaccharides are components of the outer membrane of gram-negative bacteria and have been isolated in middle ear effusions. They are known to incite significant inflammation. LPS has been shown to produce IL-1 β , IL-6, TNF- α , COX-1, and COX-2 in the middle ear [27, 55, 56]. As such, it has been used in numerous studies to induce experimental otitis media [57–59]. What is not known is how LPS induces inflammation. Eguchi et al. investigated LPS-induced cytokine production using a mouse model [60]. They showed that when using prostaglandin D₂ receptor (DP) knockout mice, LPS induced less production of IL-1 β , IL-6, and macrophage inflammatory protein (MIP)-2 (murine functional homologue to IL-8). Additionally, they demonstrated that individual administration of PGD₂ stimulates IL-1 β , IL-6, and MIP-2. They hypothesized that these data suggest a role for DP receptor antagonists as a therapeutic option in otitis media as their results suggest that LPS induces inflammation via DP [57].

Cytokine Production and Extraesophageal Reflux-Induced Otitis Media

Extraesophageal reflux has been implicated in many diseases of the aerodigestive tract, including asthma, laryngomalacia, chronic laryngitis, chronic sinusitis, and otitis media [61]. Pepsin has been identified in middle ear effusions [62]. Moreover, the pepsin concentration within the middle ear has been correlated to pharyngeal reflux episodes recorded during dual-probe pH monitoring [63]. Başoğlu et al. assessed the cytokine expression in gastric-content-induced middle ear inflammation [64]. They used a rabbit model and exposed the middle ear to the rabbit's own gastric contents via a myringotomy. They analyzed the presence of numerous cytokines via immunohistochemistry. They found significant increases in VEGF, inducible nitric oxide synthase (iNOS), endothelial nitric oxide synthase (eNOS), IL-1 β , and IL-17, implicating these cytokines as possible effectors of middle ear inflammation secondary to gastric reflux. IL-1 β is well known as a proinflammatory mediator elevated in otitis media. IL-17 is also a proinflammatory cytokine; however, its other roles are to generate Th1 responses and to provide immunity against some intracellular pathogens. To summarize, this study attempts to identify cytokine induction created by gastric content exposure. As expected, gastric contents do cause inflammation, but it is unknown if the amount of gastric exposure assessed is representative of the actual pathophysiology. Another related concern involves the presence of pepsin alone. While gastric contents create

damage, it is imperative to determine whether nonacid pepsin is capable of inducing inflammation and whether the pepsin identified in middle ear effusions is no longer exposed to a low enough concentration to be effective.

Cytokine Production in Chronic Otitis Media

Chronic middle ear inflammation with and without cholesteatoma is associated with tissue remodeling that can induce pathological changes within the middle ear space. Kuczkowski et al. performed a prospective study assessing the relationship between TNF- α , IL-1, IL-6, and IL-10 and the degree of bone destruction and level of invasion in patients with COM with and without cholesteatoma [65]. IL-10 was identified as being 5.6 and 2.6 fold greater in granulation tissue than in normal skin and cholesteatoma, respectively. The cholesteatoma expressed higher amounts of TNF- α , IL-1, and IL-6, whereas normal skin and granulation tissue showed no significant differences in these cytokines. There also was a strong inverse correlation between the IL-10 level and bone destruction and degree of invasion, suggesting a beneficial effect of IL-10. IL-10 was negatively correlated to TNF- α and IL-6. Perhaps most interestingly, however, TNF- α , IL-1, and IL-6 increased in proportion to the degree of bone destruction and invasion, confirming other studies that support their role in osteoclastic activity.

Chronic otitis media exerts its effects through pathological changes of neovascularization, sclerosis, and osteoclastogenesis. In a recent study by Sautter et al. [66], bone morphogenic protein (BMP is known to regulate osteoblastic and osteoclastic activity), fibroblast growth factor (FGF), and matrix metalloproteinases (MMP) were evaluated in an acute otitis media mouse model at various time points (1, 3, 5, and 7 days after inoculation with heat-killed *S. pneumoniae*). Their results revealed significant downregulation of various tissue remodeling cytokines (BMP and FGF) and upregulation of some members of the MMP family. Specifically, BMP 3, 4, 6, and 8a were downregulated. BMP 1 and 7 were mildly upregulated. FGF 3, 6, and r1 were downregulated, whereas MMP 2, 3, and 9 were upregulated [63]. The upregulation of MMP 2, 3, and 9 is consistent with previous studies identifying greater concentrations in mucoid middle ear effusions [67]. At this point, the specific importance of the initial downregulation of BMP and FGF family cytokines during the acute phase is not known. Conceivably, their downregulation is part of the complex interaction of molecular mediators involved in acute and chronic otitis media. Future research into the interplay between the inflammatory and tissue remodeling cytokines may provide further insight into the pathogenesis of otitis media and may offer novel ways to affect the outcome of otitis media.

Cytokine Gene Polymorphisms and Otitis Media

Historically, acute otitis media has been recognized as having high heritability [68–70]. Cytokine polymorphisms have predicted susceptibility to otitis media and the severity of respiratory syncytial viral infection [71, 72]. Additionally, the frequency of otitis media has also been correlated to cytokine polymorphisms of TNF α , IL-10, IL-6, and IFN- γ [73]. McCormick et al. recently reported a prospective longitudinal study evaluating the role of cytokine polymorphisms (TNF α ⁻³⁰⁸, IL-6⁻¹⁷⁴, and IL-1 β ⁺³⁹⁵³) and environmental risk factors for acute otitis media severity in 128 subjects [74]. They determined that disease severity was more strongly associated with tobacco smoke exposure, young age, and family history than any of the specific cytokine polymorphisms they were assessing. They did find, however, that in a subset with more severe tympanic membrane inflammation, IL-1 β ⁺³⁹⁵³ was more predictive. The fact that family history was the most significantly correlated risk factor is interesting in that it corroborates the heritability of otitis media. The lack of a significant correlation between cytokine polymorphism and acute otitis media overall implies that none exists or that the polymorphisms studied were not associated with otitis media severity of illness. It could also be the case that the disease severity is multifactorial and that family history can group many risk factors together by putting certain anatomic and molecular risks in the same cohort. Overall, this study affirms the multifactorial susceptibility of acute otitis media.

To further explore the genetics of inflammation, Rye et al. assessed sma- and Mad-related (SMAD) genes of the TGF β pathway and single-nucleotide polymorphisms (SNPs) within FBXO11 and EVI1 families and their relationship to recurrent acute otitis media or chronic otitis media with effusion in western Australians [75]. Based on studies in mice using Fbxo11, it is believed that the human analog FBXO11 is also involved in the TGF β signaling pathway [76]. Their results identified FBXO11 as being highly associated with recurrent acute otitis media and chronic otitis media. Interestingly, the single most associated SNP (rs330787) of FBXO11 that was associated with otitis media severity in this study was not associated with otitis media in the Minnesota COME/ROM family study [77].

The association of otitis media with numerous genetic variants of genes regulating cytokines offers many possibilities for future research. Learning the genetic polymorphisms of cytokines can also allow further understanding of the implications of cytokine manipulation and the subsequent effects on inflammatory and immune reactions to disease.

Conclusions

Cytokine responses in the common cold and otitis media are integral to understanding the molecular and cellular mechanisms of these diseases. Viruses causing illness in the upper respiratory tract are inextricably linked to otitis media. Within otitis media, understanding the varied effects of cytokine responses may help unlock some of the intracellular intricacies that will eventually allow better therapies for both acute and chronic otitis media.

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- Of importance
- Of major importance

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