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REVIEW

Subversion of host immune responses by otopathogens during otitis media

James M. Parrish | Manasi Soni | Rahul Mittal

Department of Otolaryngology, University of Miami Miller School of Medicine, Miami, Florida, USA

Correspondence
Rahul Mittal, Department of Otolaryngology, University of Miami-Miller School of Medicine, 1600 NW 10th Avenue, Miami, FL 33136, USA.
Email: r.mittal11@med.miami.edu

Abstract

Otitis media (OM) is one of the most common ear diseases affecting humans. Children are at greater risk and suffer most frequently from OM, which can cause serious deterioration in the quality of life. OM is generally classified into two main types: acute and chronic OM (AOM and COM). AOM is characterized by tympanic membrane swelling or otorrhea and is accompanied by signs or symptoms of ear infection. In COM, there is a tympanic membrane perforation and purulent discharge. The most common pathogens that cause AOM are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* whereas *Pseudomonas aeruginosa* and *Staphylococcus aureus* are commonly associated with COM. Innate and adaptive immune responses provide protection against OM. However, pathogens employ a wide arsenal of weapons to evade potent immune responses and these mechanisms likely contribute to AOM and COM. Immunologic evasion is multifactorial, and involves damage to host mucociliary tract, genetic polymorphisms within otopathogens, the number and variety of different otopathogens in the nasopharynx as well as the interaction between the host's innate and adaptive immune responses. Otopathogens utilize host mucin production, phase variation, biofilm production, glycans, as well as neutrophil and eosinophilic extracellular traps to induce OM. The objective of this review article is to discuss our current understanding about the mechanisms through which otopathogens escape host immunity to induce OM. A better knowledge about the molecular mechanisms leading to subversion of host immune responses will provide novel clues to develop effective treatment modalities for OM.

KEYWORDS

Otitis media, immune responses, otopathogens, chronic suppurative otitis media, *Pseudomonas aeruginosa*, defensins, phase variation

1 | INTRODUCTION

Otitis media (OM) is a serious healthcare problem in both developed and developing countries. OM refers to the inflammation of the area behind the tympanic membrane called the middle ear (Fig. 1A). The inflammatory mediators generated during OM can penetrate from middle to inner ear potentially leading to hearing loss (Fig. 1A). OM-mediated hearing loss can have serious consequences especially during childhood, including delayed language development and impaired communication. OM accounts for more than 25 million visits to physician's offices and is associated with significant healthcare costs. OM can be broadly classified into acute and chronic types. Acute otitis media (AOM) refers to any type of swelling of the tympanic membrane or otorrhea that is not attributable to otitis externa (Fig. 1B). Typically, the presentation includes inflammation of the middle ear or symptoms of infection (Fig. 1B).[1] The most common pathogens associated with AOM include *Streptococcus pneumoniae*, nontypeable *Haemophilus influenzae* (NTHi), and *Moraxella catarrhalis*.[2–6] In some cases, despite antimicrobial therapy, AOM progresses to chronic OM (COM). The most frequent type of COM is chronic suppurative OM (CSOM), characterized by tympanic membrane perforation and purulent discharge (Fig. 1B).

Host immune responses can play a crucial role in providing protection against infections including OM. Broadly, the two general types of immune defense mechanisms are innate and adaptive immune

Abbreviations: AMPs, antimicrobial peptides; AOM, acute otitis media; CSOM, chronic suppurative otitis media; COM, chronic otitis media; ETs, eosinophilic extracellular traps (ETs); HBDs, human β-defensins; LOS, lipooligosacharide; LPS, lipopolysaccharide; NETs, neutrophil extracellular traps; OM, otitis media; PAMPs, pathogen-associated molecular patterns; TNF-α, tumor necrosis factor alpha.

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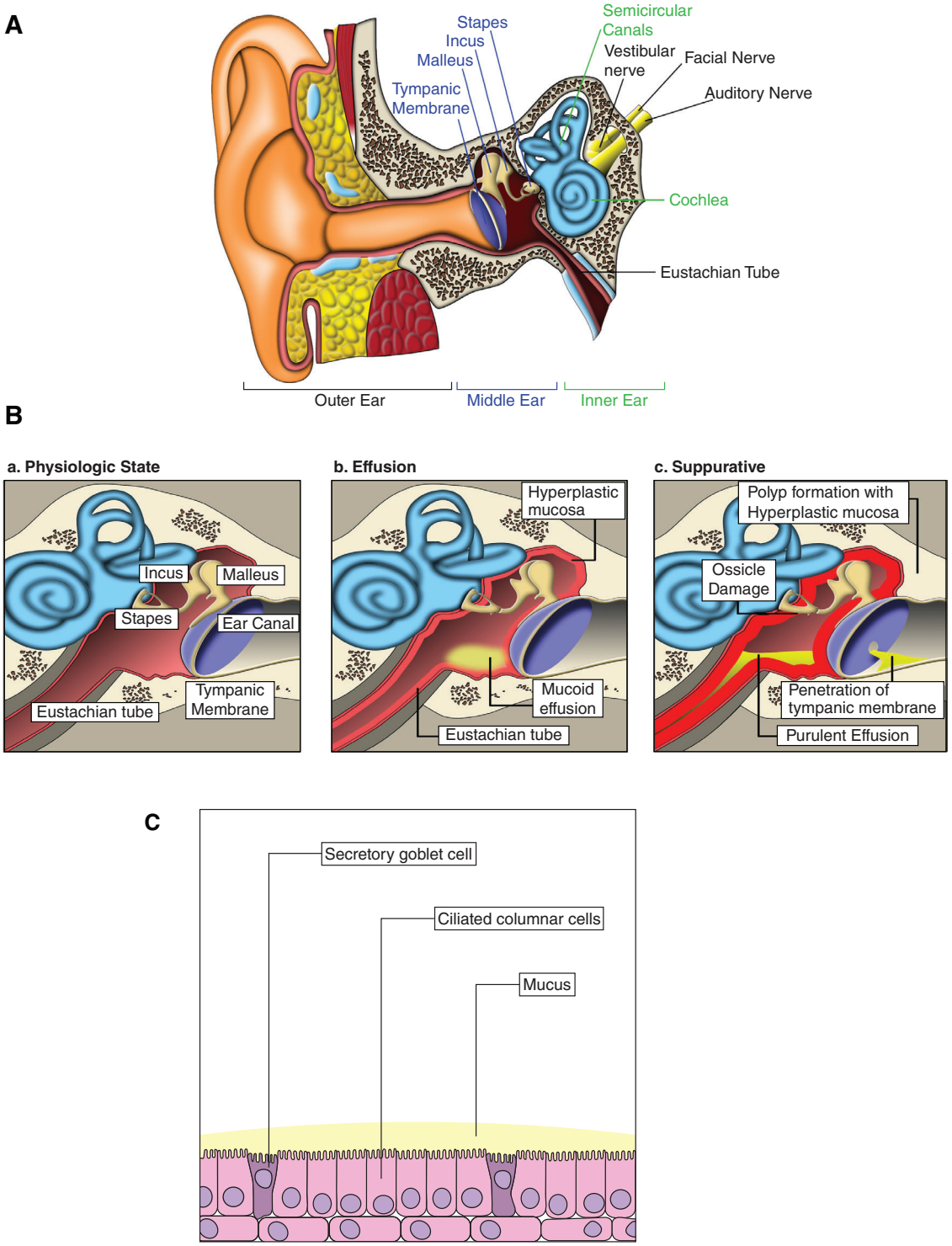

FIGURE 1 **Schematic representation of human ear, different types of otitis media (OM), and mucin production. A)** The ear is composed of three main sections: outer, middle and inner. OM refers to inflammation and/or infection of the middle ear composed of stapes, incus, and malleus as well as lined by mucosal epithelium. **B)** OM presentation: **1)** Under normal physiologic conditions, the middle ear is clear without effusion and intact tympanic membrane; **2)** However, there exists mucoid effusion and inflammation of Eustachian tube during acute OM (AOM); **3)** In chronic suppurative OM (CSOM), there is perforation of tympanic membrane and purulent discharge (adapted from Bhutta et al., [148]). **C)** The middle ear is lined with ciliated and secretory cells, covered with a thin layer of mucus (adapted from Bhutta et al., [148])

responses. Each are composed of unique but interrelated cellular and secretory components. In addition, mechanical, chemical, and microbiologic barriers, including mucin production in the middle ear (Fig. 1C), provide protection against invading pathogens. If an otopathogen manages to cross the epithelial barrier, it is then subject to recognition by neutrophils and macrophages. At times, however, the innate immune system may not be able to clear otopathogens and hence it may require lymphocytes and the adaptive immune system to halt invasion. The lymphocytes of the adaptive immune system give a more versatile defense that can provide lasting protection against subsequent reinfection from the same pathogen. The aim of this review article is to summarize the immune responses present in the middle ear and how otopathogens evade these responses to induce OM.

2 | MECHANICAL, CHEMICAL, AND MICROBIOLOGIC BARRIERS

2.1 | Eustachian tube epithelium

The Eustachian tube epithelium is versatile in its function as a mechanical, chemical, and microbiologic barrier to infection. Beyond the epithelium's basic function as a physical barrier by virtue of tight junctions, epithelial cells combine the mechanical action of mucociliary transport with the production of other chemical barriers. Some epithelial cells function to produce lysozyme, and this combines with mucoid and serous mucus from adjacent goblet cells. This mixture is antimicrobial in nature and prevents epithelial cell invasion by otopathogens. The mucociliary transport apparatus traps glycoproteins and mucus, ultimately propelling the fluid downward through the Eustachian tube and into the nasopharynx. Other innate defense mechanisms that prevent bacterial and viral pathogenicity on the epithelial surface can be described in terms of their secretory and nonsecretory nature. Importantly, secretory cells create mucins, lactotransferrin, lysozyme, defensins, and surfactants. These secretions foster a balanced, clean environment in the middle ear through both chemical and microbiologic mechanisms of action.[7–9] Hence, both flow and antimicrobial proteins provide protection against potential invasive otopathogens.[10] The middle ear also maintains inherently protective constituents including defensins, SPLUNC1, mucin, lysozymes, and TLRs.[9]

2.2 | Defensins

Defensins are members of a subset of antimicrobial peptides (AMPs). Defensins within the middle ear play a crucial role in nonenzymatic inhibition of bacteria, fungi, protozoa, and viruses.[11] Additional associations of defensins include the inhibition of bacterial toxins, proinflammatory activity that stimulates cytokine and chemokine production, as well as the creation of pores on the membranes of pathogens.[11] Three different types of defensins have been discovered, including α-, β-, and θ-defensins. Humans only express α- and β-defensins.[11]

α-Defensins are expressed in granulocytes and intestinal Paneth cells.[12] When first discovered, α-defensins were named after the former function, that is, human neutrophil peptide (HNP). Six human α-defensins have been identified; they are abbreviated as HNP1, HNP2, HNP3, HNP4, HD5, and HD6. HNPs 1–4 are expressed in granules of neutrophils, entailing an importance in immune reactions associated with OM inflammation.[13] The most potent of the α-defensins is HNP1, which appears to have activity against several strains of phagocytized NTHi.[14]

Human β-defensins (HBDs) are expressed within epithelial cells of various organs, including middle ear making them perhaps more intrinsically related to the Eustachian tube and OM. Although 11 HBDs have been discovered, only HBDs 1–4 have been extensively studied. While defensins have not been studied in large patient samples, in vitro and in vivo studies have demonstrated their induction in association with bacterial infections of the middle ear. Mouse β-defensins (mBD) 2, 3, and 4 were first noted to be up-regulated in the tubotympanums in experimental OM.[15] Of the HBDs, HBD2 is most extensively demonstrated to play a role in middle ear host immune defense.

HBD1 and HBD2 are AMPs that are capable of killing NTHi, *S. pneumoniae*, and *M. catarrhalis*.[16] Indeed, the presence of such bacteria within the middle ear is a primary source of HBD2 up-regulation. Increased levels of HBD2 have also been associated with the increased production of cytokines.[17,18] NTHi can trigger the up-regulation of IL-1α that can act synergistically with bacteria to enhance the expression of HBD2 in middle ear epithelial cells through the p38 MAP kinase pathway.[18] The increased expression of HBD-2, both at the mRNA and protein levels, has been demonstrated in inflamed middle ear mucosa from OM patients in comparison to that obtained from normal subjects.[19] In addition, the proinflammatory cytokine, IL-1α, up-regulates HBD-2 transcription via the activation of an Src-dependent Raf-MEK1/2-ERK signaling pathway in human middle ear epithelial cell line.[19] HBD2 has also shown to be up-regulated by other proinflammatory mediators such TNF-α, and LPS.[19] Finally, the recombinant human β-defensin 3 (rhBD-3) has been observed as an integral part in NTHi eradication that can be compromised through biofilm formation within the middle ear.[20]

2.3 | Surfactant and other proteins

In addition to the defensins, surfactant proteins have been found to halt infection, albeit by different mechanisms. Some of these functions include opsonization, aggregation, and phagocytosis. One of the surfactant proteins is SPLUNC1, which is a constituent of liquid covering middle ear mucosal surfaces that delivers both mechanical and antimicrobial effects against invading pathogens.[21] SPLUNC1 demonstrates broad-spectrum antimicrobial activity, while also preventing biofilm formation by organisms such as *P. aeruginosa*.[22–24] Further, as a surfactant, SPLUNC1 reduces surface tension within the upper airway and the Eustachian tube. Within a chinchilla model, however, inhibition of SPLUNC1 did not alter NHTi proliferation, while still leading to dysfunction of the Eustachian tube. It is hypothesized this dysfunction occurred as a result of decreased mucociliary clearance through the diminished antimicrobial action.

Similarly, mucins aid in the propagation of mucus cells, which creates a protective barrier. However, overproduction of mucin can

result in delayed clearance of OM pathogens, which underscores the importance of homeostasis of the middle ear.[25] Lysozymes have a slightly different defensive role. Their primary function is to destroy the bacteria's peptidoglycan cell wall.[26] Lysozymes, target bacteria by cleaving peptidoglycans at their polysaccharide backbone. As an essential piece of the bacterial cell well, glycans are frequently targeted by the host immune system. Lastly, TLRs, which can be found on the surface of many epithelial cells, provide specific immunity against pathogen-associated molecular patterns (PAMPs). PAMPs are typically unique to individual microbes.[10,27,28] As previously mentioned, should any of the potential invaders traverse through the epithelium, leukocytes, act the first line of defense after the epithelium.

3 | INNATE DEFENSES OF THE MIDDLE EAR

Besides mechanical, chemical, and microbiologic barriers, innate immunity plays a crucial role in providing protection against pathogens. Neutrophils and macrophages are an integral component of innate immunity in the middle ear and help in killing otopathogens.[9] Other cells that function as part of the initial response to infection or to foreign bodies within the middle ear include fibroblasts, mast cells, and NK cells.[9]

Overall, the innate immune system is responsible for generating the initial protective responses by triggering TLRs leading to the production of cytokines in response to invasion of the middle ear by pathogens.[29–31] The up-regulation of these cytokines can result in multiple outcomes including eradication of the infection, or can also lead to chronic otitis media (COM), which is often seen in organisms such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*.[32,33] Such organisms create biofilms, which paradoxically increase host inflammation and cytokine production that can result in COM.[34] Specific cytokines that have been associated with COM include IL-8, TNF, IL-1β, IL-6, and IFN-γ.[35,36] The inflammatory response is beneficial to a defending host because it up-regulates lymph flow, and this transports increasing quantities of antigen to the lymphoid tissue. As the antigen is transported to lymphoid tissue, it can be taken by dendritic cells that can activate adaptive immune responses.

4 | ADAPTIVE AND OTHER DEFENSES OF THE MIDDLE EAR

The activation of adaptive immune responses facilitates in clearance of pathogens from the middle ear.[37] The nasopharyngeal tonsils and adenoids play an important inductive role as components of the mucosa-associated lymphoid tissue (MALT). These areas display similarities with lymph nodes because of their role in activating an adaptive response. An important implication of these adaptive immune system sites is that they can affect both the systemic and mucosal realms of adaptive immunity, making them a particularly versatile and specific defensive barrier for invasion against incoming viruses and bacteria.

4.1 | Antibody production

The primary defensive mechanism of mucosal secretions is the immunoglobulin IgA.[38] IgA has demonstrated efficacy in protection against *Streptococcus* in human buccal epithelial cells as well as *Escherichia coli* in the urinary tract. Additionally, researchers have investigated IgA's influence in combating adherence of *S. pneumoniae* and *H. influenzae* to the nasopharynx as this specifically applies to OM. IgA in the nasopharynx has been found to be protective against OM pathogens by inhibiting adherence of bacteria to the epithelial cells. This also explains why such barriers are less effective in patients with IgA deficiency, making these individuals more prone to OM infections.[38]

4.2 | Protection via lymphocytes

T and B cells can help in providing protection against infection. At the onset of an infection, naïve $CD4^+$ T cells create memory $CD4^+$ T cells.[39–41] $CD4^+$ T cells can further be subdivided into the cytokines they respond to and produce. $CD4^+$ T cells that secrete IFN-γ are T_h1 cells, and those that release IL-4 are T_h2 cells.[42] In children, $CD4^+$ T_h cells have been demonstrated to reduce in the inhabitance of the nasopharynx by *S. pneumoniae* and *H. influenzae*.[43–46] In terms of B cell response, the primary antibodies found in the middle ear are IgA and IgG.[10] Children with fewer memory B cells have been found to be more likely to develop OM, further demonstrating the importance of B cells in immunologic protection.[47]

4.3 | Other mechanisms

Besides immunologic responses, the ear has additional protection mechanisms against microbial colonization such as defensins and other biomolecules. Commensal organisms can also provide protection against ear infections. It has been observed that although some commensal organisms, which function to preserve the synchronous organism, can perpetuate OM, others may even be protective against contracting post-upper respiratory tract infection (URTI) OM.[48] For example, *S. aureus* combats otopathogens by preventing nasopharyngeal colonization and thus may inhibit the development of OM. Similarly, *Sphingobium* may be protective against OM whereas *Bifidobacterium* has limited influence on URTI or OM.[48]

5 | IMMUNOLOGIC EVASION

Despite the presence of potential antimicrobial defense mechanisms, otopathogens can cause OM. Pathogens employ a wide arsenal of weapons to evade potent immune responses and induce OM. Examples of such evasion mechanisms include coexisting viral infections resulting in a negative pressure buildup in the Eustachian tube, exaggerated cytokine production leading to increased inflammation, and a build-up of mucin, which decreases mucociliary clearance and hence results in OM infections that are less likely to be cleared (Table 1).[49,50] Bacteria are even able to manipulate their gene expression through phase variation and interact with other pathogens, increasing their virulence and

TABLE 1 A summary of evasion strategies employed by otopathogens to subvert host immune responses

Evasion strategy	Mechanism	References
Viral Infection and Eustachian tube dysfunction	Viral infection can serve as a catalyst for inflammation in the Eustachian tube. Viral inflammation prevents the normal function of mucociliary flow and lysozyme proteins that typically eliminate bacteria. The result is dysfunctional negative pressure in the middle ear, enabling previously colonized organisms to evade normal defenses.	Avadhanula et al., 2006 Pittet, Hall-Stoodley, Rutkowski, 2010
Cytokines	Increased viruses and bacteria in the middle ear are associated with inflammatory mediators such as histamine, leukotriene B_4 and IL-8, all of which prevent the efficacy of antibiotics. Additional cytokines such as IL-2, IL-10, TGF-β, IL-4, IL-5, CCL3, and G-CSF released by viruses cause tissue damage and subsequent bacterial infection.	Avadhanula et al., 2006 Bakaletz, 2010 Canafax et al., 1998 Chonmaitree et al., 1994 Chonmaitree et al., 1996 Jossart et al., 1994 Smirnova, Birchall, and Pearson, 2004
Mucin	Mucus is the initial barrier in the middle ear for protection from viruses and bacteria. Mucins form the mucus layer, saturate the cilia, and facilitate the mucociliary transportation clearance system. However, exaggerated mucin lead to bacterial retention and hampers mucociliary clearance. Specific mucins (MUC2, MUC5AC, and MUC5B) have been correlated with the pathogenesis of OM and evasion of immune responses by otopathogens.	Precaiado et al., 2010 Samuels et al., 2017
Phase variation	Some bacterial pathogens are also able to employ phase variation to regulate gene expression and evade host immune responses. NTHi phase variation creates a rearrangement of glycosyltransferase genes, allele on/off switching of N^6-adenine DNA methyltransferase (ModA), and manipulation of the polythymidine (poly-T) tract in the *hia* promoter. Phase variation enables pathogens to modify their genetic makeup to both obtain nutrients from their environment, and to resist oxidative stress from the host immune system. Phase variation also affects biofilm formation.	Apicella et al., 2018 Borrelli et al., 1999 Brookman et al., 2016 Wren at al., 2014
Polymicrobial infections	OM commonly infects the middle ear after a viral URI, resulting in diminished antibiotic response and penetration. Viruses also create a more viscous mucous, in addition to releasing cytokines that prolong the course of OM.	Giebink, 1989 Canafax et al., 1998 Chonmaitree et al., 1996 Bakaletz, 2010
Biofilms	Biofilms found on bacteria give pathogens increased resistance to being cleared, hence leading to chronic OM. Pathogens such as *S. pneumoniae* and *H. influenzae* have biofilms that enable avoidance of complement immunity and phagocytosis.	Pang and Swords, 2017 Andre et al., 2017 Cuevas et al., 2017 Das et al., 2017 Domenech et al., 2013 Jurcisek et al., 2017 Marti et al., 2017 Tikhomirova and Kidd, 2013
Glycans	Glycans have unique evasion mechanisms. Not only do they prevent complement activation, but they also apply molecular mimicry and commensal interactions to evade host cell detection.	Comstock and Kasper, 2006
Neutrophils	Neutrophils are the first line of host defense against infections and form "neutrophil extracellular traps" (NETs). NETs and fibrin, which are often the primary mode of defense, are inhibited from being released by respiratory pathogens, such as *S. pneumoniae* and *S. aureus*. NETs can also contribute to extracellular DNA (eDNA) that can promote biofilm formation and subversion of host immune responses. NETs can also contribute to thicker effusion.	Schachern et al., 2017 Val et al., 2016
Eosinophils	Eosinophilic extracellular traps (ETs) are more often seen with eosinophilic OM. Their function is to release eosinophilic granules and DNA traps to destroy pathogens. ETs can also contribute to thicker effusion.	Hurst and Venge, 2000 Ueki et al., 2016 Ueki et al., 2017

decreasing the chances of host clearance.[51–53] Immunologic evasion is multifactorial, and often depends on damage to the host mucociliary tract, genetic regulatory changes within otopathogens, the number as well as a variety of different otopathogens that have colonized the nasopharynx, and finally, the delicate interplay between the host's adaptive and innate immune response (Table 1).

5.1 | Viral infections and evasion through Eustachian tube dysfunction

It is estimated that 94% of AOM cases are preceded by either the "common cold" or another URTI.[54] In order of importance, such viral infections include: respiratory syncytial virus (RSV), rhinovirus, adenovirus, coronavirus, bocavirus, influenza virus, parainfluenza virus, enterovirus, and human metapneumovirus.[10,55] Typically, children have asymptomatic bacterial colonization of the nasopharynx. However, a viral infection can initiate inflammation within the Eustachian tube. Although the epithelium's normal mucociliary flow and lysozyme proteins readily eliminate bacteria under normal circumstances, this host protection can be stymied by new virally induced inflammation. This inflammation of the Eustachian tube precipitates a dysfunctional negative pressure within the middle ear that allows previously colonized organisms to evade the epithelium's normal defenses. This negative pressure often occurs with greater severity in

children less than 24 months old in comparison to children from 25 to 47 months of age.

5.2 | Up-regulation of cytokines by otopathogens

Although cytokine production is often explained as a directed action by a host immune system, several examples suggest that the secretion of chemokines and cytokines can be manipulated by otopathogens for enhanced survival. For example, viral infections are associated with Eustachian tube dysfunction, which is at least partially due to their up-regulation of cytokine production and through their mediation of the inflammatory response. More recently it has been hypothesized that inflammatory mediators could facilitate bacterial adherence and colonization. With rising levels of inflammation, a number of epithelial cell surface antigens increase, and many of these are known to serve as sites for bacterial receptors.[49,56]

Increased levels of live viruses and bacteria in the middle ear are associated with mediators of the inflammatory response, including histamine, leukotriene B_4 and IL-8, all of which are known to hinder the delivery and ultimate penetration of antibiotics.[57–60] The cytokines such as IL-2, IL-10, TGF-β, IL-4, IL-5, and G-CSF induced by viral infections can cause tissue damage making a fertile ground for bacterial infection.[61]

Although many of these cytokines appear to be induced by viral infections, and potentially later manipulated by bacterial pathogens, this is not always the case. CCL3, for example, is one cytokine that is known to be a potent OM inflammation effector and it also appears to have a predominantly protective role in mice models. CCL3 knockout mice have been shown to have higher pathogenic colonization rates and a defect in host macrophages, resulting in reduced ability of the host to combat *P. aeruginosa*. Such knowledge of the pervasive role of CCL3 could help in developing therapeutic strategies to treat persistent OM infections.[62]

5.3 | Mucin

Damage to the host mucociliary transport system of the middle ear is thought to be the initial event that facilitates evasion of Eustachian tube defenses by otopathogens. Mucus is the first barrier that protects the epithelium from viruses and bacteria. Secreted by goblet cells within the middle ear epithelium, mucins form a mucus layer that saturates the cilia and enable the mucociliary transportation clearance system of Eustachian tube (Fig. 1C). The production of mucins is normally limited to the orifice of the Eustachian tube and the areas immediately surrounding the area. The normal physiologic levels of mucin promote the clearance of microbes. However, exaggerated production of mucus leads to entrapment of bacteria preventing their clearance and enhances the retention of bacteria leading to OM. Specific mucins have also been correlated with various types of OM. For example, MUC5B has been found to be the most common mucin involved in COM.[63] OM with effusion has demonstrated the presence of mucin 2 (MUC2), mucin 5AC (MUC5AC), and mucin 5B (MUC5B).[64]

When left untreated, AOM may progress to chronic OM with effusion (COME). Specifically, NTHi is a common bacterial pathogen that contributes to this pathology. Although researchers continue to investigate NTHi's pathogenic mechanism, it is known that NTHi activates *MUC5AC* mucin transcription once the bacterial cell has been disturbed.[65] The initial step required to stimulate MUC5AC transcription is the triggering of p38 mitogen-activated protein kinase. Conversely, a negative feedback mechanism exists in which phosphoinositide 3-kinase-Akt pathway leads to inactivation of NTHi-influenced *MUC5AC* transcription by communicating with p38 mitogen-activated protein kinase pathway.[65] Ultimately, the activation of this pathway can lead to an overabundance of mucin, thereby contributing to conductive hearing loss in COME, decreased mucociliary clearance, increasing the bacterial retention in the middle ear and persistent infection.[65] Further, it has recently been discovered that curcumin, the principal curcuminoid of turmeric (*Curcuma longa*), is an inhibitor of NTHi-associated MUC5AC production.[66] The molecular mechanisms by which cucurmin down-regulates MUC5AC transcription is through the manipulation of AP-1, a transcription factor in the MAPK pathway. Cucurmin has the ability to impede NTHi-MUC5A expression by down-regulating MKK3/6 activation of p38 MAPK and by up-regulating MKP-1.[66] Further studies are warranted to explore the therapeutic potential of curcumin for OM.

5.4 | Pathogen phase variation

Besides mucin, many bacterial pathogens are also able to use phase variation to regulate gene expression and evade potent host immune responses (Fig. 2), though researchers are still attempting to better elucidate this phenomenon.[52,67–69] NTHi, in particular, has various forms of phase variation such as rearrangement and modification of glycosyltransferase genes, allele on/off switching of N^6-adenine DNA methyltransferase (ModA), and manipulation of the polythymidine (poly-T) tract in the *hia* promoter.[51,52,67,70] NTHi ModA allele phaseversion was studied in animal models that specifically investigated OM. It has been demonstrated that animals with the "on" modA2 phaseversion had a higher burden of disease.[51–53] Tetranucleotide repeats that influence phase variation in lipo-oligosaccharide genes have also been studied. It was observed that an "on" to "off" switch of the *oafA* gene can provide an overall benefit in the middle ear.[71]

NTHi is also known for *hia* transcription in its promotor region, and Hia protein, which is another form of phase variation.[70] As previously alluded to, *hia* is able to modify its poly-T tract. The overall objective of poly-T tract variation is to escape opsonophagocytic killing by the host immune system. It has been demonstrated that strains of NTHi with less *hia* expression are more successful at evasion of the host.[70] Phase variation also allows species of pathogens to manipulate their genetic makeup to obtain nutrients from their environment as well as to resist oxidative stress from host immune system.[52] Phase variation affects biofilm formation and can prolong its formation for a longer period of time leading to immune system evasion.[52] Findings also suggest increased biofilm formation under alkaline conditions at pH of 9. Further, biofilms formed in alkaline environments have an associated increase in HMW adhesins in the *modA2* ON phase version group.[52] *ModA2* ON is also associated with greater susceptibility to oxidative stress and less resistance to neutrophil-directed killing.[72]

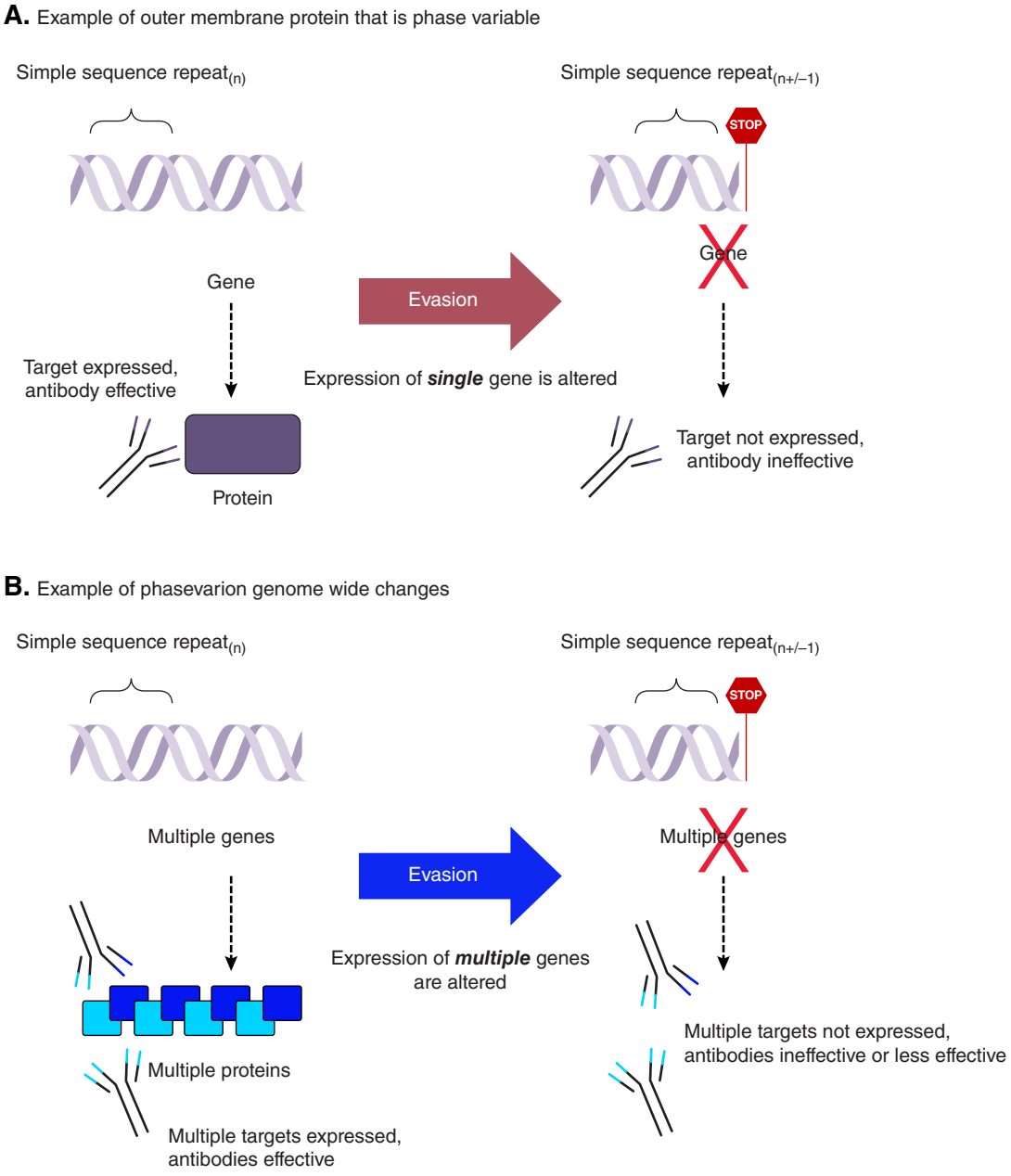

FIGURE 2 **Phase variation in bacteria. A)** The presence of simple sequence repeats (SSR) in outer membrane proteins of otopathogens leads to simple strand mispairing during genome replication. This causes alteration in DNA sequence and consequently "OFF/ON" expression of selected proteins. Due to unavailability of selected protein during "OFF" expression, antibody against the target is not able to recognize it leading to evasion of potent immune responses. **B)** Otopathogens can employ phasevariome genome variation leading to alteration of multiple genes and proteins. Antibodies are no longer able to recognize or bind with very low affinity to altered proteins leading to subversion of host immune responses

Protection against oxidative stress is also seen in other OM pathogens, such as *S. pneumoniae* by manipulation of the thiol-specific antioxidant (TlpA/TSA), which is an additional example of host immune evasion.[73]

Such phase variation can also be seen amongst other OM pathogens such as *M. cararrhalis*.[74–80] Three DNA methyltransferase (ModM) alleles, modM1-3, have been found in *M. catarrhalis* that can affect pathogenesis and recovery.[75] Phase variation in *S. pneumoniae* (specifically transparent [T] variants) demonstrate differences in host evasion based specifically on avoidance of complement-mediated destruction. It has been hypothesized that the transparent T variant specifically enables better adhesion to the environment of the nasopharynx.[74,76,81,82] In human experimental models with tympanostomy tube placement, there was increased expression of NanA, HylA, and PspA in transparent T variants. T variants also had increased levels of NanA and HylA at baseline, suggesting increased virulence in these variants.[77] These findings suggest that the T variant of the pneumococcal pathogen is responsible for the pathogenesis of OM.[81]

5.5 | Polymicrobial infections

In addition to phase variation, otopathogens take advantage of other existing infections to induce OM. This phenomenon typically involves

viral coinfections and often makes OM a polymicrobial disease.[56] The most common viruses associated with OM are influenza virus, parainfluenza virus, rhinovirus, coronavirus, and RSV.[55,56] Most frequently, OM occurs 2–5 day after a viral URI.[83] This coinfection results in poor antibiotic response, which is hypothesized to be due to reduced penetration of the middle ear in virus-infected children.[60] An additional theory is that the inflammatory mediators released by viruses result in delayed resolution of OM.[58] Animal models have shown that polymicrobial infection additionally results in hypersecretion and increased viscosity of mucus.[56] Similar findings have been seen among humans with influenza A infections. However, in humans, the biochemical quality of the secretions is modified as opposed to changes that occur simply in the quantity or viscosity of mucus.[84] Changes in secretions in human mucosa are thought to be due to viral neuraminidase.[85] Such polymicrobial interactions increase the virulence of pathogens while evading the host responses.

5.6 | Biofilm formation

Biofilms provide an additional immunologic evasion mechanism. Beyond increasing bacterial adherence and pathogenicity, biofilms have been found to be integral in the pathogenesis of OM.[86–89] Biofilms have been demonstrated to contribute to the inability of the host immune system to clear bacteria during COM.[90] Specifically, biofilms created by *S. pneumoniae* and *H. influenzae* appear to help these pathogens to avoid complement immunity and phagocytosis.[91–97] *S. pneumoniae* has many different protein variants (namely, PspA, PspC, and Phts e PLY) that provide protection against complement-mediated microbial killing.[93] Such bacterial proteins interweave in a complex interplay of reactions to inhibit complement from adequately protecting the host. For example, PspA is a factor that appears to be helpful in halting CRP and C3 convertase formation. PspC also appears to inhibit C3 convertase though by different target molecules, including, FH, C4BP, and Vitronectin. Finally, the Ply factor targets C1q, IgG, and L-Ficolin, which keeps the host complement system away from the bacterial cells. Virulence peptide 1 (*vp1*) perpetuates biofilm development in the middle ear.[96] At the current time, *vp1* is hypothesized to detect local amino acid levels. However, the precise molecular mechanisms through which *vp1* influences biofilms and virulence are still not known.

Besides bacterial proteins, extracellular DNA (eDNA) and associated DNABII have been hypothesized to be involved in biofilm architecture and structural integrity.[94,98,99] eDNA itself is a structural component of the extracellular polymeric substance. The eDNA is stabilized by eDNA strands, forming a meshwork of crisscrossing strands. In *H. Influenzae*, isogenic mutants (ΔcomE) have demonstrated a decreased presence of eDNA and type IV pilus (Tfp) in silico. The nonisogenic mutant, however, demonstrated elevated levels of fractal structures, which appears to have a role in enabling biofilm nutrient exchange, and feedback mechanisms. Extracellular RNA also plays an important role in the initial steps of biofilm synthesis, though its overall function is less important.[100] Overall, the biofilm synthesized by NTHi has been found to release extracellular DNA and a β -glucan.[100]

In addition to eDNA, the other genetic association related to biofilm formation in NTHi involves the activation of lipooligosaccharides (LOS), which are located on the surface of bacteria. Phosphorylcholine (PCho) is found on some LOS and it has been found that PCho+ aids in stabilizing NTHi biofilms in animal models.[101] NTHi pathogenicity can be further enhanced by mutations in *luxS*. This influences the quorum signaling pathway and can result in the development as well as the establishment of biofilms.[102] Biofilms can be particularly pathogenic in the case of OM, as the infections are polymicrobial and can hence create synergistic reactions, increased growth of organisms, antimicrobial tolerance, increased virulence and persistence, as well as exaggerated levels of exopolysaccharide (EPS).[88,100,103–110] Biofilms have been found to be associated with adenoid hypertrophy and middle ear effusion.[88] Syntrophy may also occur, which supports the idea that the growth of one product increases the growth of another, contributing to infections with elevated pathogenicity.[111] A specific example of this is a coinfection with NanA influenza and *S. pneumoniae*. NanA is required for adherence of influenza as well as for the interplay of pathogenicity between the two organisms.[110]

5.7 | Glycans

Glycans are carbohydrate structures that are found on the surfaces of pathogen and host structures, and they are often involved in highly specific interactions.[112] Bacteria present glycosylated molecules to their host. These molecules include polysaccharides, glycoproteins, LOS, and LPS. Pathogens utilize the glycans that are found on the surface of many host cells as sources of carbon,[113] as bacterial toxin targets,[114,115] and as locations of attachment and subsequent invasion.[116,117] One such example is *H. influenzae*, which has been shown to obtain sialic acid from host cells in the inner ear of animal models. The host-acquired sialic acid is then used for catabolism and sialylation of the pathogen LOS.[118–120] Sialic acid catabolism and the genes associated with this process (*nanEK*, *nanA*, *siaA*, *nagBA*) have been demonstrated to be integral to the pathogenicity of *H. influenzae*.[121]

Changes in the bacterial capsular structure often underlie bacterial evasion of the host immune response. In some cases, these structures are inhibitory against immune functions of the host.[122] Other mechanisms include bacteria that create surface glycosylation structures that mimic host glycans. In many cases this allows for subversion of host immune system recognition. This mechanism helps microbes avoid immune defenses such as macrophages recognition that, in particular, utilize glycan surfaces to recognize both gram-positive and gram-negative bacteria.

Capsular polysaccharides have also been observed to mimic glycan structures on host surfaces.[122] A common mechanism that is illustrative of this concept involves variations of the group A Streptococcus (GAS) capsule. Hyaluronan (HA) is expressed on the GAS capsule. It has been observed that HA interacts with CD44 on host cells and that high molecular weight HA leads to decreased phagocytosis by macrophages. By comparison, low molecular weight HA leads to increased macrophage uptake.

Some bacteria alter protein glycosylation patterns and avoid cleavage by host proteases. NTHi appears to use glycosylation to protect its surface-exposed high molecular weight adhesin 1 (HMW1A).[123,124] This action serves to attach HMW1A to the bacterial surface along with protecting it from degradation by the host.

NTHi peptidoglycans and an associated outer membrane protein (OMP) P6, serve as a ligand for TLR2.[125] Likewise, LOS is a ligand for TLR2 and TLR4. As one might expect, polymorphisms associated with OMP P6 or LOS have been found to be associated with recurrent cases of OM.[126] An important clinical implication of these findings is that patient groups with chronic middle ear infections such as CSOM appear to have lower baseline levels of protein and mRNA related to TLR2, TLR4, and TLR6.[127]

Commensal organisms often make use of glycan interactions with host cells, which provides for more long-standing avoidance techniques.[112] *Neisseria meningitidis*, for example is a pathogen that is commensal in the nasopharynx of an estimated 5–10% of all humans.[128,129] The *N. meningitidis* serogroup C conjugate (MCC) vaccine achieves protection by generating bactericidal antibodies that target the serogroup C capsule.[130] Even with the use of the MCC vaccine, at least three *N. meningitidis* escape strains have been noted. The *N. meningitidis* resistance against the vaccine appears to be related to changes in capsular production. Resistance has been associated with the insertion sequence (IS), IS1301. IS1301 lies within the intergenic region of the *sia* and *ctr* operons. The addition of the IS results in increased capsular polysaccharide synthesis. Aside from the vaccine resistance that is attained through increased capsular production, the increased polysaccharide also has been observed to interfere with complement activation. Because sialic acid-containing capsules can interfere with complement cascade amplification, the alternative pathway is inhibited on the bacterial surface and consequent decreases of C3 and membrane attack complex have also been observed. Although modification of the glycans located on the surface of pathogens assists in the aforementioned evasion mechanisms, modification of host proteins has also been noted.

Bacteria can modify host glycans using enzymes such as glycosyltransferases and glycosidases.[122] Of these enzymes, the most commonly studied are the neuraminidases. *S. pneumoniae* has been observed to produce at least 10 glycosidases, 3 of which are neuraminidases that are integral in nutrient acquisition and pathogenesis, for example, N- acetylneuraminate lyase (NanA), sialidase B (NanB), and neuramidase C (NanC).[113] *S. pneumoniae* uses these enzymes for critical aspects of colonization such as utilizing carbon sources on host mucosal surfaces and in generating biofilm architecture. *S. pneumoniae* has been associated with induction of infection secondary to influenza A. The influenza A neuraminidase activity on sialylated structures appears to facilitate the *S. pneumoniae* secondary infection.[110]

The methods by which glycans can be modified to evade the host immune system are diverse. Such mechanisms include the use of host carbohydrate structures as sources of carbon, targeting host glycans with pathogen toxins, using polysaccharides as sites of attachment or as an opportunity for mimicry of host structures.[122] As molecular mimicry via glycan modification can impact either innate or adaptive immune function, investigation of these interactions could pave the way for the development of future therapies.

5.8 | Neutrophils and eosinophils

OM pathogens are also able to escape host defenses by manipulating the host's innate immune system. Neutrophils are typically the first responders and form neutrophil extracellular traps (NETs) to kill pathogens. In AOM, animal models demonstrated that NETs and fibrin formation are initial host defenses in middle ear infections.[131–133] However, a persistence of NETs and fibrin contribute to biofilm formation, and hence chronic disease and effusion.[131] This can be attributed to the fact that NETs lead to a thicker effusion, which creates a more fertile ground for biofilm formation.[132] The end result is an association between NETs, COM and middle ear effusions.[133] Respiratory pathogens, such as *S. pneumoniae* and *S. aureus* appear to prevent the release of NETs, at least in part due to nucleases impeding antibacterial proteins and breaking down the NETs.[134,135] Although NETs have been more extensively studied in pulmonary diseases as targets for therapy,[136,137] their role in OM, especially in CSOM, is still not clear and warrants further studies.

In addition to NETs, eosinophilic extracellular traps (ETs) have been implicated in the pathogenesis of OM. ETs are associated with eosinophilic OM (EOM) and appear to facilitate the release of eosinophilic granules as well as DNA traps to destroy pathogens.[138–141] Eosinophils and mast cells, which both contribute to T_h2 host cell immunity, are often found in COM infections.[141] Eosinophils in the middle ear are activated by cytokines such as IL-5 and eotaxin. Eventually, eosinophils undergo extracellular trap cell death, also known as ETosis.[140] ETosis involves cell death that results in the creation of extracellular traps (ETs), which play an important role in eosinophilic OM.[140] The mechanism by which these eosinophilic traps promote evasion of immunity by otopathogens is through an increased amount of eosinophilic secretions, resulting in increased viscosity.[128] This increased viscosity will hinder the clearance of otopathogens by the mucocilary system and will also prevent the penetration of antimicrobial compounds. Furthermore, the end product of ETosis is the expulsion of proteins, cytokines, chromatins, and lipid mediators, all of which are components of inflammation, perpetuating damage of surrounding tissue.[129] These findings suggest that fibrin, NETs, and ETs could serve as a potential avenue for therapy against OM.[131]

6 | IMMUNE EVASION BY COM PATHOGENS SPECIFICALLY OTOPATHOGENIC *P. AERUGINOSA*

The precise mechanisms underlying the pathogenesis of COM are still far from clear. There is a need to initiate research studies in this area. A few studies have started to decipher the molecular mechanisms that can lead to COM/CSOM. Otopathogenic *P. aeruginosa* has been demonstrated to activate the PKC pathway by phosphorylation of PKC-alpha leading to the invasion of human middle ear epithelial cells. The ability

of otopathogenic *P. aeruginosa* to activate PKC pathway is dependent on OprF expression. The results of this study demonstrate the crucial role that the PKC pathway and OprF expression plays in the pathogenesis of COM/CSOM.[142] The PKC pathway is responsible for the activation of many subsequent signaling cascades that can further activate the expression of proinflammatory cytokines. Specifically, cytokines such as TNF-α and IL-1β can contribute to chronic inflammation.[143,144] It has also been demonstrated that otopathogenic *P. aeruginosa* enters and multiplies inside human and mouse primary macrophages that is dependent on both microtubule and actin dependent processes.[145] The colonization of macrophages by otopathogenic *P. aeruginosa* will lead to evasion of potent immune responses and may contribute to persistence of infection observed during CSOM. COM/CSOM is also associated with increased biofilm formation, which further explains why COM infections are so difficult to treat.[146] However, it remains to be seen how COM pathogens such as *P. aeruginosa* and *S. aureus* manipulates host immunity to promote biofilm formation in the middle ear leading to subversion of immune responses and induced infection.

7 | HOST GENETIC VARIABILITY IN SUSCEPTIBILITY TO OM

APC tumor suppressor gene deletions have also been associated with COM. For example, COM can result in an abnormal skin growth composed of stratified squamous keratinized epithelium known clinically as cholesteatoma. Children with cholesteatomas have genetic associations with a deletion in the APC tumor suppressor gene, as well as variations in the connexin gap-junction genes, *GJB2* and *GJB6*.[144] These studies highlight the need for understanding the role of genetics in predisposition to COM/CSOM and in the evasion of host immune responses.

8 | CONCLUSION AND FUTURE DIRECTIONS

OM, one of the most common childhood infections, is characterized by mucus overproduction and elevated levels of inflammation within the middle ear. Common pathogens such as *S. pneumoniae*, *H. influenzae* and *Moraxella*, are capable of inducing OM due to a complex interplay between pathogens and host immunity, ultimately leading to evasion of potent immune responses. A wide variety of mechanisms such as biofilm formation, phase variation, and glycans have been implicated in subversion of host immune responses by AOM pathogens. Biofilms are composed of extracellular substance that forms a protective layer, which shields the pathogens from the host immune system as well as the interventional therapies. Identification and characterization of proteins such as DNABII and Type IV pili (Tfp) has led to the development of antibodies that target such structures, which subsequently has demonstrated the destruction of biofilms that are protective to *H. influenzae*.[147] Although targeting either of these proteins independently has subtle yet different effects, such strategies supports the idea that eradication of biofilm formation from an "outside-in" approach may assist in clearing both adherent and planktonic generations of NTHi.

Although destruction of the biofilm structure may facilitate pathogen vulnerability, pharmaceutic interventions targeting intracellular pathways have also demonstrated potential as a therapeutic strategy for OM. Understanding the role of host pathways such as p38 MAPK and MAPK phosphatase MKP-1 as well as mucin signaling cascades and how natural compounds such as curcumin is able to target these pathways may help in developing novel treatment modalities for OM. Further investigation of such therapies is warranted, particularly those focused on increasing local drug concentration and improving targeted delivery within the middle ear.

In addition to biofilm formation and host pathways, phase variation allows bacterial pathogens to regulate their genetic makeup to escape destruction from the host immune system. Research has shown that ModA phase variation specifically gives NTHi pathogens the ability to evade host immunity, increasing pathogenesis and virulence.[52] Further research focused on ModA phase variation of NTHi may allow for genetic manipulation to prevent and treat such infections. NTHi also has adhesins (type IV fimbriae) and Hia that help protect it from the host immune system. Further investigation of these structures and how NTHi infects host middle ear could be useful to create a vaccine. This type of vaccine could be used to prevent future OM infections by synthesizing multiple adhesin types.[70]

Glycans are an additional extracellular component of bacterial pathogens that are an important structural component in the cell wall. As such, glycans have unique membrane proteins that can even be acquired from the host cell. Due to the varied nature of bacterial glycans and the role they play in evasion of the host immune system, it is possible that future research may allow for the modification of glycans as defense mechanism against OM pathogens.[112]

Although a number of studies have highlighted the molecular mechanisms involved in subversion of immune response during AOM, our knowledge regarding COM is still very limited. Despite the high prevalence of COM, especially CSOM in both developed and developing countries, it is still an underexplored research area. There is an urgent need to perform research studies determining the molecular mechanisms employed by COM/CSOM pathogens such as *P. aeruginosa* to cause middle ear infection. Understanding the role of host immune cells and NETs in the pathophysiology of COM/CSOM will provide novel insights into the pathogenesis of the disease. As with other aspects of immunologic host evasion, focusing research on integral mechanisms of host evasion and resistance is likely to accelerate the overall knowledge, prevention, and treatment of COM/CSOM. The availability of novel effective treatment modalities beyond antibiotic therapy will lead to improved quality of life of many OM patients and their families.

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AUTHORSHIP

J.M.P. and M.S. contributed equally to this work. All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

1. Lieberthal AS, Carroll AE, Chonmaitree T, et al. The diagnosis and management of acute otitis media. *Pediatrics*. 2013;131:e964-e999.
2. LaCross NC, Marrs CF, Gilsdorf JR. Population structure in nontypeable *Haemophilus influenzae*. *Infect Genet Evol*. 2013;14:125-136.
3. Segal N, Leibovitz E, Dagan R, et al. Acute otitis media-diagnosis and treatment in the era of antibiotic resistant organisms: updated clinical practice guidelines. *Int J Pediatr Otorhinolaryngol*. 2005;69:1311-1319.
4. Vergison A. Microbiology of otitis media: a moving target. *Vaccine*. 2008;26 Suppl 7:G5-G10.
5. Chi DH, Hendley JO, French P, et al. Nasopharyngeal reservoir of bacterial otitis media and sinusitis pathogens in adults during wellness and viral respiratory illness. *Am J Rhinol*. 2003;17:209-214.
6. Bakaletz LO, Novotny LA. Nontypeable *Haemophilus influenzae* (NTHi). *Trends Microbiol*. 2018;26:727-728.
7. Lim JH, Jono H, Koga T, et al. Tumor suppressor CYLD acts as a negative regulator for non-typeable Haemophilus influenza-induced inflammation in the middle ear and lung of mice. *PLoS One*. 2007;2:e1032.
8. McGuire JF. Surfactant in the middle ear and eustachian tube: a review. *Int J Pediatr Otorhinolaryngol*. 2002;66:1-15.
9. Mittal R, Kodiyan J, Gerring R, et al. Role of innate immunity in the pathogenesis of otitis media. *Int J Infect Dis*. 2014;29:259-267.
10. Schilder AG, Chonmaitree T, Cripps AW, et al. Otitis media. *Nat Rev Dis Primers*. 2016;2:16063.
11. Yang D, Liu ZH, Tewary P, et al. Defensin participation in innate and adaptive immunity. *Curr Pharm Des*. 2007;13:3131-3139.
12. Park MS, Kim JI, Lee I, et al. Towards the Application of Human Defensins as Antivirals. *Biomol Ther (Seoul)*. 2018;26:242-254.
13. Wilson SS, Wiens ME, Smith JG. Antiviral mechanisms of human defensins. *J Mol Biol*. 2013;425:4965-4980.
14. Bishop-Hurley SL, Schmidt FJ, Erwin AL, et al. Peptides selected for binding to a virulent strain of *Haemophilus influenzae* by phage display are bactericidal. *Antimicrob Agents Chemother*. 2005;49:2972-2978.
15. Jin Shin D, Gan-Undram S, Jin Kim S, et al. Expression of beta-defensins in the tubotympanum of experimental otitis media. *Acta Otolaryngol*. 2006;126:1040-1045.
16. Lee HY, Andalibi A, Webster P, et al. Antimicrobial activity of innate immune molecules against *Streptococcus pneumoniae*, *Moraxella catarrhalis* and nontypeable *Haemophilus influenzae*. *BMC Infect Dis*. 2004;4:12.
17. Underwood M, Bakaletz L. Innate immunity and the role of defensins in otitis media. *Curr Allergy Asthma Rep*. 2011;11:499-507.
18. Moon SK, Lee HY, Pan H, et al. Synergistic effect of interleukin 1 alpha on nontypeable *Haemophilus influenzae*-induced up-regulation of human beta-defensin 2 in middle ear epithelial cells. *BMC Infect Dis*. 2006;6:12.
19. Moon SK, Lee HY, Li JD, et al. Activation of a Src-dependent Raf-MEK1/2-ERK signaling pathway is required for IL-1alpha-induced upregulation of beta-defensin 2 in human middle ear epithelial cells. *Biochim Biophys Acta*. 2002;1590:41-51.
20. Jones EA, McGillivary G, Bakaletz LO. Extracellular DNA within a nontypeable *Haemophilus influenzae*-induced biofilm binds human beta defensin-3 and reduces its antimicrobial activity. *J Innate Immun*. 2013;5:24-38.
21. McGillivary G, Bakaletz LO. The multifunctional host defense peptide SPLUNC1 is critical for homeostasis of the mammalian upper airway. *PLoS One*. 2010;5:e13224.
22. Di YP. Functional roles of SPLUNC1 in the innate immune response against Gram-negative bacteria. *Biochem Soc Trans*. 2011;39:1051-1055.
23. Gakhar L, Bartlett JA, Penterman J, et al. PLUNC is a novel airway surfactant protein with anti-biofilm activity. *PLoS One*. 2010;5:e9098.
24. Liu Y, Di ME, Chu HW, et al. Increased susceptibility to pulmonary *Pseudomonas* infection in Splunc1 knockout mice. *J Immunol*. 2013;191:4259-4268.
25. Preciado D, Burgett K, Ghimbovschi S, et al. NTHi induction of Cxcl2 and middle ear mucosal metaplasia in mice. *Laryngoscope*. 2013;123:E66-E71.
26. Parker D, Prince A. Innate immunity in the respiratory epithelium. *Am J Respir Cell Mol Biol*. 2011;45:189-201.
27. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity*. 2011;34:637-650.
28. Kumar S, Ingle H, Prasad DV, et al. Recognition of bacterial infection by innate immune sensors. *Crit Rev Microbiol*. 2013;39:229-246.
29. Trune DR, Kempton B, Hausman FA, et al. Correlative mRNA and protein expression of middle and inner ear inflammatory cytokines during mouse acute otitis media. *Hear Res*. 2015;326:49-58.
30. Leichtle A, Lai Y, Wollenberg B, et al. Innate signaling in otitis media: pathogenesis and recovery. *Curr Allergy Asthma Rep*. 2011;11:78-84.
31. Murphy TF, Chonmaitree T, Barenkamp S, et al. Panel 5: microbiology and immunology panel. *Otolaryngol Head Neck Surg*. 2013;148:E64-E89.
32. Kurabi A, Pak K, Ryan AF, et al. Innate Immunity: orchestrating inflammation and resolution of otitis media. *Curr Allergy Asthma Rep*. 2016;16:6.
33. Morris P. Chronic suppurative otitis media. *BMJ Clin Evid*. 2012;2012.
34. Mittal R, Lisi CV, Gerring R, et al. Current concepts in the pathogenesis and treatment of chronic suppurative otitis media. *J Med Microbiol*. 2015;64:1103-1116.
35. Si Y, Zhang ZG, Chen SJ, et al. Attenuated TLRs in middle ear mucosa contributes to susceptibility of chronic suppurative otitis media. *Hum Immunol*. 2014;75:771-776.
36. Elmorsy S, El-Naggar MM, Abdel aal SM, et al. Sinus aspirates in chronic rhinosinusitis: fungal colonization of paranasal sinuses, evaluation of ICAM-1 and IL-8 and studying of immunological effect of long-term macrolide therapy. *Rhinology*. 2010;48:312-317.
37. Massa HM, Lim DJ, Kurono Y, et al. Chapter 101 - Middle Ear and Eustachian Tube Mucosal Immunology. In: J Mestecky, W Strober, MW Russell, BL Kelsall, H Cheroutre, BN Lambrecht, eds. *Mucosal Immunology* (4th ed.). Boston, MA: Academic Press; 2015:1923-1942.
38. Kurono Y, Shimamura K, Shigemi H, et al. Inhibition of bacterial adherence by nasopharyngeal secretions. *Ann Otol Rhinol Laryngol*. 1991;100:455-458.

39. McKinstry KK, Strutt TM, Swain SL. The potential of CD4 T-cell memory. *Immunology*. 2010;130:1-9.
40. Kelly DF, Pollard AJ, Moxon ER. Immunological memory: the role of B cells in long-term protection against invasive bacterial pathogens. *JAMA*. 2005;294:3019-3023.
41. Pichichero ME. Booster vaccinations: can immunologic memory outpace disease pathogenesis? *Pediatrics*. 2009;124:1633-1641.
42. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today*. 1996;17:138-146.
43. Sharma SK, Pichichero ME. Cellular immune response in young children accounts for recurrent acute otitis media. *Curr Allergy Asthma Rep*. 2013;13:495-500.
44. Sharma SK, Roumanes D, Almudevar A, et al. CD4+ T-cell responses among adults and young children in response to *Streptococcus pneumoniae* and *Haemophilus influenzae* vaccine candidate protein antigens. *Vaccine*. 2013;31:3090-3097.
45. Mureithi MW, Finn A, Ota MO, et al. T cell memory response to pneumococcal protein antigens in an area of high pneumococcal carriage and disease. *J Infect Dis*. 2009;200:783-793.
46. Zhang Q, Bagrade L, Bernatoniene J, et al. Low CD4 T cell immunity to pneumolysin is associated with nasopharyngeal carriage of pneumococci in children. *J Infect Dis*. 2007;195:1194-1202.
47. Sharma SK, Casey JR, Pichichero ME. Reduced serum IgG responses to pneumococcal antigens in otitis-prone children may be due to poor memory B-cell generation. *J Infect Dis*. 2012;205:1225-1229.
48. Chonmaitree T, Jennings K, Golovko G, et al. Nasopharyngeal microbiota in infants and changes during viral upper respiratory tract infection and acute otitis media. *PLoS One*. 2017;12:e0180630.
49. Avadhanula V, Rodriguez CA, Devincenzo JP, et al. Respiratory viruses augment the adhesion of bacterial pathogens to respiratory epithelium in a viral species- and cell type-dependent manner. *J Virol*. 2006;80:1629-1636.
50. Pittet LA, Hall-Stoodley L, Rutkowski MR, et al. Influenza virus infection decreases tracheal mucociliary velocity and clearance of *Streptococcus pneumoniae*. *Am J Respir Cell Mol Biol*. 2010;42:450-460.
51. Atack JM, Srikhanta YN, Fox KL, et al. A biphasic epigenetic switch controls immunoevasion, virulence and niche adaptation in nontypeable *Haemophilus influenzae*. *Nat Commun*. 2015;6:7828.
52. Brockman KL, Jurcisek JA, Atack JM, et al. ModA2 phasevarion switching in nontypeable *Haemophilus influenzae* increases the severity of experimental otitis media. *J Infect Dis*. 2016;214:817-824.
53. Brockman KL, Azzari PN, Branstool MT, et al. Epigenetic regulation alters biofilm architecture and composition in multiple clinical isolates of nontypeable *Haemophilus influenzae*. *MBio*. 2018;9.
54. Nokso-Koivisto J, Marom T, Chonmaitree T. Importance of viruses in acute otitis media. *Curr Opin Pediatr*. 2015;27:110-115.
55. Chonmaitree T, Revai K, Grady JJ, et al. Viral upper respiratory tract infection and otitis media complication in young children. *Clin Infect Dis*. 2008;46:815-823.
56. Bakaletz LO. Immunopathogenesis of polymicrobial otitis media. *J Leukoc Biol*. 2010;87:213-222.
57. Jossart GH, Canafax DM, Erdmann GR, et al. Effect of *Streptococcus pneumoniae* and influenza A virus on middle ear antimicrobial pharmacokinetics in experimental otitis media. *Pharm Res*. 1994;11:860-864.
58. Chonmaitree T, Patel JA, Sim T, et al. Role of leukotriene B4 and interleukin-8 in acute bacterial and viral otitis media. *Ann Otol Rhinol Laryngol*. 1996;105:968-974.
59. Chonmaitree T, Patel JA, Lett-Brown MA, et al. Virus and bacteria enhance histamine production in middle ear fluids of children with acute otitis media. *J Infect Dis*. 1994;169:1265-1270.
60. Canafax DM, Yuan Z, Chonmaitree T, et al. Amoxicillin middle ear fluid penetration and pharmacokinetics in children with acute otitis media. *Pediatr Infect Dis J*. 1998;17:149-156.
61. Smirnova MG, Birchall JP, Pearson JP. The immunoregulatory and allergy-associated cytokines in the aetiology of the otitis media with effusion. *Mediators Inflamm*. 2004;13:75-88.
62. Deniffel D, Nuyen B, Pak K, et al. Otitis media and nasopharyngeal colonization in CCL3(-/-) Mice. *Infect Immun*. 2017;85.
63. Preciado D, Goyal S, Rahimi M, et al. MUC5B Is the predominant mucin glycoprotein in chronic otitis media fluid. *Pediatr Res*. 2010;68:231-236.
64. Samuels TL, Yan JC, Khampang P, et al. Association of gel-forming mucins and aquaporin gene expression with hearing loss, effusion viscosity, and inflammation in otitis media with effusion. *JAMA Otolaryngol Head Neck Surg*. 2017;143:810-817.
65. Wang B, Lim DJ, Han J, et al. Novel cytoplasmic proteins of nontypeable *Haemophilus influenzae* up-regulate human MUC5AC mucin transcription via a positive p38 mitogen-activated protein kinase pathway and a negative phosphoinositide 3-kinase-Akt pathway. *J Biol Chem*. 2002;277:949-957.
66. Konduru AS, Matsuyama S, Lee BC, et al. Curcumin inhibits NTHi-induced MUC5AC mucin overproduction in otitis media via upregulation of MAPK phosphatase MKP-1. *Int J Inflam*. 2017;2017:4525309.
67. Apicella MA, Coffin J, Ketterer M, et al. Nontypeable *Haemophilus influenzae* lipooligosaccharide expresses a terminal ketodeoxyoctanoate in vivo, which can be used as a target for bactericidal antibody. *mBio*. 2018;9.
68. Wren JT, Blevins LK, Pang B, et al. Influenza A virus alters pneumococcal nasal colonization and middle ear infection independently of phase variation. *Infect Immun*. 2014;82:4802-4812.
69. Borrelli S, Camou T, Hortal M, et al. Frequencies of lipopolysaccharide-defined epitopes in *Haemophilus influenzae* type b and non-typable isolates determined with monoclonal antibodies. *Clin Microbiol Infect*. 1999;5:364-370.
70. Atack JM, Winter LE, Jurcisek JA, et al. Selection and counterselection of hia expression reveals a key role for phase-variable expression of Hia in infection caused by nontypeable *Haemophilus influenzae*. *J Infect Dis*. 2015;212:645-653.
71. Fox KL, Atack JM, Srikhanta YN, et al. Selection for phase variation of LOS biosynthetic genes frequently occurs in progression of nontypeable *Haemophilus influenzae* infection from the nasopharynx to the middle ear of human patients. *PLoS One*. 2014;9:e90505.
72. Brockman KL, Branstool MT, Atack JM, et al. The ModA2 phasevarion of nontypeable *Haemophilus influenzae* regulates resistance to oxidative stress and killing by human neutrophils. *Sci Rep*. 2017;7:3161.
73. Andisi VF, Hinojosa CA, de Jong A, et al. Pneumococcal gene complex involved in resistance to extracellular oxidative stress. *Infect Immun*. 2012;80:1037-1049.
74. Li Q, Li YX, Douthitt K, et al. Role of the alternative and classical complement activation pathway in complement mediated killing against *Streptococcus pneumoniae* colony opacity variants during acute pneumococcal otitis media in mice. *Microbes Infect*. 2012;14:1308-1318.
75. Blakeway LV, Power PM, Jen FE, et al. ModM DNA methyltransferase methylome analysis reveals a potential role for *Moraxella catarrhalis* phasevarions in otitis media. *FASEB J*. 2014;28:5197-5207.
76. Arai J, Hotomi M, Hollingshead SK, et al. *Streptococcus pneumoniae* isolates from middle ear fluid and nasopharynx of children with

acute otitis media exhibit phase variation. *J Clin Microbiol*. 2011;49:1646-1649.

77. Li-Korotky HS, Lo CY, Banks JM. Interaction of pneumococcal phase variation, host and pressure/gas composition: virulence expression of NanA, HylA, PspA and CbpA in simulated otitis media. *Microb Pathog*. 2010;49:204-210.
78. McEllistrem MC, Ransford JV, Khan SA. Characterization of in vitro biofilm-associated pneumococcal phase variants of a clinically relevant serotype 3 clone. *J Clin Microbiol*. 2007;45:97-101.
79. Long JP, Tong HH, Shannon PA, et al. Differential expression of cytokine genes and inducible nitric oxide synthase induced by opacity phenotype variants of *Streptococcus pneumoniae* during acute otitis media in the rat. *Infect Immun*. 2003;71:5531-5540.
80. Tong HH, Weiser JN, James MA, et al. Effect of influenza A virus infection on nasopharyngeal colonization and otitis media induced by transparent or opaque phenotype variants of *Streptococcus pneumoniae* in the chinchilla model. *Infect Immun*. 2001;69:602-606.
81. Li-Korotky HS, Lo CY, Zeng FR, et al. Interaction of phase variation, host and pressure/gas composition: pneumococcal gene expression of PsaA, SpxB, Ply and LytA in simulated middle ear environments. *Int J Pediatr Otorhinolaryngol*. 2009;73:1417-1422.
82. Li-Korotky HS, Banks JM, Lo CY, et al. Interaction of pneumococcal phase variation and middle ear pressure/gas composition: an in vitro model of simulated otitis media. *Microb Pathog*. 2008;45:201-206.
83. Giebink GS. The microbiology of otitis media. *Pediatr Infect Dis J*. 1989;8:S18-S20.
84. Doyle WJ, Skoner DP, White M, et al. Pattern of nasal secretions during experimental influenza virus infection. *Rhinology*. 1996;34:2-8.
85. Hirano T, Kurono Y, Ichimiya I, et al. Effects of influenza A virus on lectin-binding patterns in murine nasopharyngeal mucosa and on bacterial colonization. *Otolaryngol Head Neck Surg*. 1999;121:616-621.
86. Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis*. 2002;8:881-890.
87. Heukelekian H, Heller A. relation between food concentration and surface for bacterial growth. *J Bacteriol*. 1940;40:547-558.
88. Tawfik SA, Ibrahim AA, Talaat IM, et al. Role of bacterial biofilm in development of middle ear effusion. *Eur Arch Otorhinolaryngol*. 2016;273:4003-4009.
89. Van Hoecke H, De Paepe AS, Lambert E, et al. *Haemophilus influenzae* biofilm formation in chronic otitis media with effusion. *Eur Arch Otorhinolaryngol*. 2016;273:3553-3560.
90. Pang B, Swords WE. *Haemophilus parainfluenzae* strain ATCC 33392 forms biofilms *in vitro* and during experimental otitis media infections. *Infect Immun*. 2017;85.
91. Domenech M, Ramos-Sevillano E, Garcia E, et al. Biofilm formation avoids complement immunity and phagocytosis of *Streptococcus pneumoniae*. *Infect Immun*. 2013;81:2606-2615.
92. Tikhomirova A, Kidd SP. *Haemophilus influenzae* and *Streptococcus pneumoniae*: living together in a biofilm. *Pathog Dis*. 2013;69:114-126.
93. Andre GO, Converso TR, Politano WR, et al. Role of *Streptococcus pneumoniae* proteins in evasion of complement-mediated immunity. *Front Microbiol*. 2017;8:224.
94. Das J, Mokrzan E, Lakhani V, et al. Extracellular DNA and type IV pilus expression regulate the structure and linetics of biofilm formation by montypeable *Haemophilus influenzae*. *mBio*. 2017;8.
95. Jurcisek JA, Brockman KL, Novotny LA, et al. Nontypeable *Haemophilus influenzae* releases DNA and DNABII proteins via a T4SS-like complex and ComE of the type IV pilus machinery. *Proc Natl Acad Sci U S A*. 2017;114:E6632-E6641.
96. Cuevas RA, Eutsey R, Kadam A, et al. A novel streptococcal cell-cell communication peptide promotes pneumococcal virulence and biofilm formation. *Mol Microbiol*. 2017;105:554-571.
97. Marti S, Puig C, Merlos A, et al. Bacterial lysis through interference with peptidoglycan synthesis increases biofilm formation by nontypeable *Haemophilus influenzae*. *mSphere*. 2017;2.
98. Devaraj A, Buzzo J, Rocco CJ, et al. The DNABII family of proteins is comprised of the only nucleoid associated proteins required for nontypeable *Haemophilus influenzae* biofilm structure. *Microbiologyopen*. 2018;7:e00563.
99. Idicula WK, Jurcisek JA, Cass ND, et al. Identification of biofilms in post-tympanostomy tube otorrhea. *Laryngoscope*. 2016;126:1946-1951.
100. Domenech M, Pedrero-Vega E, Prieto A, et al. Evidence of the presence of nucleic acids and beta-glucan in the matrix of non-typeable *Haemophilus influenzae* in vitro biofilms. *Sci Rep*. 2016;6:36424.
101. Hong W, Mason K, Jurcisek J, et al. Phosphorylcholine decreases early inflammation and promotes the establishment of stable biofilm communities of nontypeable *Haemophilus influenzae* strain 86-028NP in a chinchilla model of otitis media. *Infect Immun*. 2007;75:958-965.
102. Armbruster CE, Hong W, Pang B, et al. LuxS promotes biofilm maturation and persistence of nontypeable *Haemophilus influenzae* in vivo via modulation of lipooligosaccharides on the bacterial surface. *Infect Immun*. 2009;77:4081-4091.
103. Dzink JL, Tanner AC, Haffajee AD, et al. Gram negative species associated with active destructive periodontal lesions. *J Clin Periodontol*. 1985;12:648-659.
104. Kreth J, Merritt J, Qi F. Bacterial and host interactions of oral streptococci. *DNA Cell Biol*. 2009;28:397-403.
105. Moore WE, Holdeman LV, Smibert RM, et al. Bacteriology of severe periodontitis in young adult humans. *Infect Immun*. 1982;38:1137-1148.
106. Syed SA, Loesche WJ. Bacteriology of human experimental gingivitis: effect of plaque age. *Infect Immun*. 1978;21:821-829.
107. Egland PG, Palmer RJ Jr., Kolenbrander PE. Interspecies communication in *Streptococcus gordonii-Veillonella atypica* biofilms: signaling in flow conditions requires juxtaposition. *Proc Natl Acad Sci U S A*. 2004;101:16917-16922.
108. Kononen E, Paju S, Pussinen PJ, et al. Population-based study of salivary carriage of periodontal pathogens in adults. *J Clin Microbiol*. 2007;45:2446-2451.
109. Yadav MK, Chae SW, Go YY, et al. *In vitro* multi-species biofilms of methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* and their host interaction during *in vivo* colonization of an otitis media rat model. *Front Cell Infect Microbiol*. 2017;7:125.
110. Wren JT, Blevins LK, Pang B, et al. Pneumococcal neuraminidase A (NanA) promotes biofilm formation and synergizes with influenza A virus in nasal colonization and middle ear infection. *Infect Immun*. 2017;85.
111. Ramsey MM, Rumbaugh KP, Whiteley M. Metabolite cross-feeding enhances virulence in a model polymicrobial infection. *PLoS Pathog*. 2011;7:e1002012.
112. Comstock LE, Kasper DL. Bacterial glycans: key mediators of diverse host immune responses. *Cell*. 2006;126:847-850.
113. King SJ. Pneumococcal modification of host sugars: a major contributor to colonization of the human airway? *Mol Oral Microbiol*. 2010;25:15-24.
114. Deng L, Song J, Gao X, et al. Host adaptation of a bacterial toxin from the human pathogen *Salmonella* Typhi. *Cell*. 2014;159:1290-1299.

115. Shewell LK, Harvey RM, Higgins MA, et al. The cholesterol-dependent cytolysins pneumolysin and streptolysin O require binding to red blood cell glycans for hemolytic activity. *Proc Natl Acad Sci U S A*. 2014;111:E5312-E5320.
116. De Oliveira DM, Hartley-Tassell L, Everest-Dass A, et al. Blood group antigen recognition via the group A streptococcal M protein mediates host colonization. *mBio*. 2017;8.
117. Rossez Y, Gosset P, Boneca IG, et al. The lacdiNAc-specific adhesin LabA mediates adhesion of *Helicobacter pylori* to human gastric mucosa. *J Infect Dis*. 2014;210:1286-1295.
118. Vimr ER, Kalivoda KA, Deszo EL, et al. Diversity of microbial sialic acid metabolism. *Microbiol Mol Biol Rev*. 2004;68:132-153.
119. Bouchet V, Hood DW, Li J, et al. Host-derived sialic acid is incorporated into *Haemophilus influenzae* lipopolysaccharide and is a major virulence factor in experimental otitis media. *Proc Natl Acad Sci U S A*. 2003;100:8898-8903.
120. Allen S, Zaleski A, Johnston JW, et al. Novel sialic acid transporter of *Haemophilus influenzae*. *Infect Immun*. 2005;73:5291-5300.
121. Haines-Menges BL, Whitaker WB, Lubin JB, et al. Host sialic acids: a delicacy for the pathogen with discerning taste. *Microbiol Spectr*. 2015;3:10.1128/microbiolspec.MBP-0005-2014.
122. Poole J, Day CJ, von Itzstein M, et al. Glycointeractions in bacterial pathogenesis. *Nat Rev Microbiol*. 2018;16:440-452.
123. Grass S, Buscher AZ, Swords WE, et al. The *Haemophilus influenzae* HMW1 adhesin is glycosylated in a process that requires HMW1C and phosphoglucomutase, an enzyme involved in lipooligosaccharide biosynthesis. *Mol Microbiol*. 2003;48:737-751.
124. Gross J, Grass S, Davis AE, et al. The *Haemophilus influenzae* HMW1 adhesin is a glycoprotein with an unusual N-linked carbohydrate modification. *J Biol Chem*. 2008;283:26010-26015.
125. Chen R, Lim JH, Jono H, et al. Nontypeable *Haemophilus influenzae* lipoprotein P6 induces MUC5AC mucin transcription via TLR2–TAK1-dependent p38 MAPK-AP1 and IKKβ-IκBα-NF-κB signaling pathways. *Biochem Biophys Res Commun*. 2004;324:1087-1094.
126. Emonts M, Veenhoven RH, Wiertsema SP, et al. Genetic polymorphisms in immunoresponse genes TNFA, IL6, IL10, and TLR4 are associated with recurrent acute otitis media. *Pediatrics*. 2007;120:814-823.
127. Si Y, Zhang ZG, Chen SJ, et al. Attenuated TLRs in middle ear mucosa contributes to susceptibility of chronic suppurative otitis media. *Human Immunol*. 2014;75:771-776.
128. Serruto D, Spadafina T, Ciucchi L, et al. Neisseria meningitidis GNA2132, a heparin-binding protein that induces protective immunity in humans. *Proc Natl Acad Sci U S A*. 2010;107:3770-3775.
129. Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis*. *Lancet*. 2007;369:2196-2210.
130. Uria MJ, Zhang Q, Li Y, et al. A generic mechanism in *Neisseria meningitidis* for enhanced resistance against bactericidal antibodies. *J Exp Med*. 2008;205:1423-1434.
131. Schachern PA, Kwon G, Briles DE, et al. Neutrophil extracellular traps and fibrin in otitis media: analysis of human and chinchilla temporal bones. *JAMA Otolaryngol Head Neck Surg*. 2017;143:990-995.
132. Thornton RB, Wiertsema SP, Kirkham LA, et al. Neutrophil extracellular traps and bacterial biofilms in middle ear effusion of children with recurrent acute otitis media–a potential treatment target. *PLoS One*. 2013;8:e53837.
133. Val S, Poley M, Brown K, et al. Proteomic characterization of middle ear fluid confirms neutrophil extracellular traps as a predominant innate immune response in chronic otitis media. *PLoS One*. 2016;11:e0152865.
134. Storisteanu DM, Pocock JM, Cowburn AS, et al. Evasion of neutrophil extracellular traps by respiratory pathogens. *Am J Respir Cell Mol Biol*. 2017;56:423-431.
135. Short KR, von Kockritz-Blickwede M, Langereis JD, et al. Antibodies mediate formation of neutrophil extracellular traps in the middle ear and facilitate secondary pneumococcal otitis media. *Infect Immun*. 2014;82:364-370.
136. Porto BN, Stein RT. Neutrophil extracellular traps in pulmonary diseases: too much of a good thing? *Front Immunol*. 2016;7:311.
137. Cortjens B, van Woensel JB, Bem RA. Neutrophil extracellular traps in respiratory disease: guided anti-microbial traps or toxic webs? *Paediatr Respir Rev*. 2017;21:54-61.
138. Ueki S, Konno Y, Takeda M, et al. Eosinophil extracellular trap cell death-derived DNA traps: Their presence in secretions and functional attributes. *J Allergy Clin Immunol*. 2016;137:258-267.
139. Ueki S, Tokunaga T, Fujieda S, et al. Eosinophil ETosis and DNA traps: a new look at eosinophilic inflammation. *Curr Allergy Asthma Rep*. 2016;16:54.
140. Ueki S, Ohta N, Takeda M, et al. Eosinophilic otitis media: the aftermath of eosinophil extracellular trap cell death. *Curr Allergy Asthma Rep*. 2017;17:33.
141. Hurst DS, Venge P. Evidence of eosinophil, neutrophil, and mast-cell mediators in the effusion of OME patients with and without atopy. *Allergy*. 2000;55:435-441.
142. Mittal R, Grati M, Yan D, et al. *Pseudomonas aeruginosa* Activates PKC-Alpha to invade middle ear epithelial cells. *Front Microbiol*. 2016;7:255.
143. Redig AJ, Platanias LC. The protein kinase C (PKC) family of proteins in cytokine signaling in hematopoiesis. *J Interferon Cytokine Res*. 2007;27:623-636.
144. Jensen RG, Johansen HK, Bjarnsholt T, et al. Recurrent otorrhea in chronic suppurative otitis media: is biofilm the missing link? *Eur Arch Otorhinolaryngol*. 2017;274:2741-2747.
145. Mittal R, Lisi CV, Kumari H, et al. Otopathogenic *Pseudomonas aeruginosa* enters and survives inside macrophages. *Front Microbiol*. 2016;7:1828.
146. Jennings BA, Prinsley P, Philpott C, et al. The genetics of cholesteatoma. A systematic review using narrative synthesis. *Clin Otolaryngol*. 2018;43:55-67.
147. Novotny LA, Jurcisek JA, Ward MO Jr., et al. Antibodies against the majority subunit of type IV pili disperse nontypeable *Haemophilus influenzae* biofilms in a LuxS-dependent manner and confer therapeutic resolution of experimental otitis media. *Mol Microbiol*. 2015;96:276-292.
148. Bhutta MF, Thornton RB, Kirkham LS, Kerschner JE, Cheeseman MT. Understanding the aetiology and resolution of chronic otitis media from animal and human studies. *Dis Model Mech*. 2017;10(11):1289-1300.

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