

Review

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Dysregulation of immune response in otitis media

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Abstract

Objective. Otitis media (OM) is a common reason for children to be prescribed antibiotics and undergo surgery but a thorough understanding of disease mechanisms is lacking. We evaluate the evidence of a dysregulated immune response in the pathogenesis of OM.

Methods. A comprehensive systematic review of the literature using search terms [otitis media OR glue ear OR AOM OR OME] OR [middle ear AND (infection OR inflammation)] which were run through Medline and Embase via Ovid, including both human and animal studies. In total, 82 955 studies underwent automated filtering followed by manual screening. One hundred studies were included in the review.

Results. Most studies were based on in vitro or animal work. Abnormalities in pathogen detection pathways, such as Toll-like receptors, have confirmed roles in OM. The aetiology of OM, its chronic subgroups (chronic OM, persistent OM with effusion) and recurrent acute OM is complex; however, inflammatory signalling mechanisms are frequently implicated. Host epithelium likely plays a crucial role, but the characterisation of human middle ear tissue lags behind that of other anatomical subsites.

Conclusions. Translational research for OM presently falls far behind its clinical importance. This has likely hindered the development of new diagnostic and treatment modalities. Further work is urgently required; particularly to disentangle the respective immune pathologies in the clinically observed phenotypes and thereby work towards more personalised treatments.

Introduction

Otitis media (OM) is an umbrella term for inflammation of the middle ear and is a leading cause of medical consultations, antibiotic prescriptions and surgery in children (Ref. 1). Worldwide there are in excess of 700 million cases of acute OM per year, with the majority occurring in children under 5 years old. Most children will be affected by OM at some point; over 60% of children will have experienced at least one episode of acute OM by their first birthday, and more than 15% will have suffered recurrent acute episodes (Ref. 2).

Most patients exhibit complete resolution of infective symptoms of acute OM within a few days. However, a subgroup subsequently suffers from ongoing pathology, including (1) recurrent acute OM, in which there are regular episodes of acute infection causing pain but with resolution of symptoms in between. (2) A persistent fluid effusion in the middle ear (OM with effusion; OME); this impairs the conduction of sound through the middle ear which leads to hearing loss and is associated with a negative impact on language acquisition, behaviour and progress at school (Ref. 3). (3) Chronic OM (COM); of which there are two subtypes – mucosal and squamous. Mucosal COM indicates an inflamed middle ear with an associated tympanic membrane (TM) perforation. Mucosal COM can be either active (producing discharge) or inactive (dry). Squamous COM is caused by severe retraction of the TM and is associated with a buildup of keratin (known as cholesteatoma) which can be locally erosive. These terms are summarised in the attached glossary (Box 1). These subgroups are not exclusive and may overlap and change with time, or indeed lead to one another.

Despite the considerable burden of disease, it presently remains unclear why the majority of children make a spontaneous recovery from limited episodes of acute OM but a subset, who are frequently otherwise apparently immunocompetent, develop recurrent acute or chronic disease.

In health, the human immune system regulates the response to infectious stimuli by providing robust action against non-self-antigens whilst exerting tolerance to the host's own cells. However, at many anatomical sites, dysfunction of this process drives many inflammatory disease processes (Ref. 4). Here we review the evidence of this dysregulation in the transition from sporadic uncomplicated acute OM to recurrent acute or chronic forms of OM. We suggest that understanding this process will be key to developing novel therapeutics, which have remained largely unchanged for over 50 years.

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Box 1. Glossary of terms The following terms are based upon definitions provided in (Ref. 1) and (Ref. 100).

Otitis media (OM)	An umbrella term for inflammation of the middle ear.
Acute otitis media (AOM)	Rapid onset signs and symptoms of middle ear inflammation.
Recurrent acute otitis media (rAOM)	≥3 discrete episodes of AOM in a 6-month period OR ≥4 discrete episodes of AOM over 12 months with at least one episode in the last 6 months
Otitis media with effusion (OME)	Fluid within the middle ear but no signs or symptoms of AOM
Glue ear	Analogous term to otitis media with effusion (OME).
Chronic otitis media (COM)	Middle ear inflammation with persistent or recurrent ear discharge. Can be subdivided into mucosal and squamous subtypes. Mucosal COM has inflamed middle ear mucosa associated with a perforated tympanic membrane (TM) and can be active (producing discharge) or inactive (dry). Squamous COM occurs due to retraction of the TM and is associated with cholesteatoma formation.
Cholesteatoma	Abnormal accumulation of keratin in the middle ear, which can be locally destructive.
Chronic suppurative otitis media (CSOM)	Analogous term to active mucosal COM.

Methods

A comprehensive systematic review of the literature using search terms [otitis media OR glue ear OR AOM OR OME] OR [middle ear AND (infection OR inflammation)] were run through Medline and Embase via Ovid. Both human and animal studies were included. In total, 82 955 studies underwent automated filtering for the English language only articles, full-text availability only, dated 1990–2021. Following de-duplication, 2557 abstracts were manually screened. One hundred studies were included in the review, including those identified in bibliographies of returned studies for a comprehensive literature review. A summary PRISMA diagram is available in Supplementary materials.

Pathogen detection in the middle ear

Acute and recurrent acute otitis media

An initial episode of acute OM may be viral or bacterial in nature, and the former can predispose to the latter (Ref. 5). Clinically it is not immediately apparent which is the causative pathogen in each case; however, a bacterial species is identified in approximately two-thirds of sampled middle ears in the acute setting (Ref. 6). The most common bacterial otopathogens in acute OM are shown in Table 1.

The origin of these organisms is likely from the nasopharynx via the Eustachian tube (Fig. 1), as demonstrated by studies which show no significant difference in microbiome diversity between nasal washings and middle ear effusion during an episode of acute OM (Ref. 7). One theory is that initial viral infection of the nasopharynx leads to an inflammatory reaction in the Eustachian tube, causing Eustachian tube dysfunction, and translocation of bacteria into the middle ear cavity (Refs 8, 9). This is

Table 1. Typical bacterial species in acute otitis media; adapted from Mather *et al.* (Ref. 6)

Bacterial species	Prevalence
<i>Streptococcus pneumoniae</i>	0.30 (CI 0.27–0.32)
<i>Haemophilus influenzae</i>	0.23 (CI 0.20–0.26)
<i>Moraxella catarrhalis</i>	0.05 (CI 0.04–0.06)

also consistent with the notion that the Eustachian tube is shorter and more horizontal in children than adults, which could facilitate bacterial translocation and contribute to their relatively higher risk of OM.

In order to generate an immune response, the host must first detect the presenting pathogen. This is mediated by pattern-recognition receptors (PRRs) such as membrane-bound Toll-like receptors (TLRs) or C-type lectins which are expressed on diverse host cell types including epithelial cells, as well as classical immune sentinels such as macrophages and dendritic cells. They detect invariant microbial motifs or pathogen-associated molecular patterns (PAMPs) in the extracellular space and trigger signal transduction via adaptor proteins such as myeloid differentiation primary response protein 88 (MyD88), leading to a pro-inflammatory response (Ref. 10).

Multiple defects in TLR signalling (Fig. 2) have been described in animal models of OM. *Tlr9* knockout models show a prolonged inflammatory response in the middle ear and delayed bacterial clearance (Ref. 11), and *Tlr4* knockout models have reduced capacity for TNF production, a late increase in *IL-10* expression, and failure to eradicate bacteria (Ref. 12). Similarly, *Tlr2* knockout models demonstrate a more severe OM phenotype compared with wildtypes – including a higher potential for systemic sepsis. They also have reduced macrophage recruitment and produce fewer pro-inflammatory mediators, which is hypothesised to lead to impaired bacterial clearance (Refs 13, 14, 15). Whilst the majority of studies on TLR signalling in OM have been conducted in mice, polymorphisms in *TLR4* have also been observed clinically in children with a propensity towards recurrent acute OM in population-based studies (Ref. 16).

Functional signal transduction proteins downstream from TLRs, such as myeloid differentiation primary response gene 88 (MyD88) and Tir-domain-containing adaptor inducing interferon β (TRIF), have been shown to be essential for resolution of OM in mice (Refs 17, 18). Similarly, patients with mutations in *MyD88* and *IRAK4*, which is also part of TLR-mediated signal transduction, have a tendency towards recurrent AOM (Ref. 19). The multiple positive findings in this pathway together suggest defective TLR-mediated pathogen detection and subsequent signal transduction impairs the host response to infection in the middle ear cleft.

However, even in situations of fully functional TLRs, it has been identified that otopathogens themselves have mechanisms to overcome TLR-mediated detection; for example, *Moraxella catarrhalis* has been shown to deliver outer membrane vesicles in response to TLR2 binding, and surface proteins – such as ubiquitous surface protein A1 (UspA1) – modulate the inflammatory response of epithelial cells. It therefore seems that otopathogens can interact with specific TLRs to subdue the host response to infection once they have infiltrated the tissue, making them challenging to eradicate (Ref. 20).

Otitis media with effusion

Most research efforts to date have focused on pathogen detection in recurrent AOM and defects in these pathways are much less clearly described in OME. Indeed, it remains unclear whether

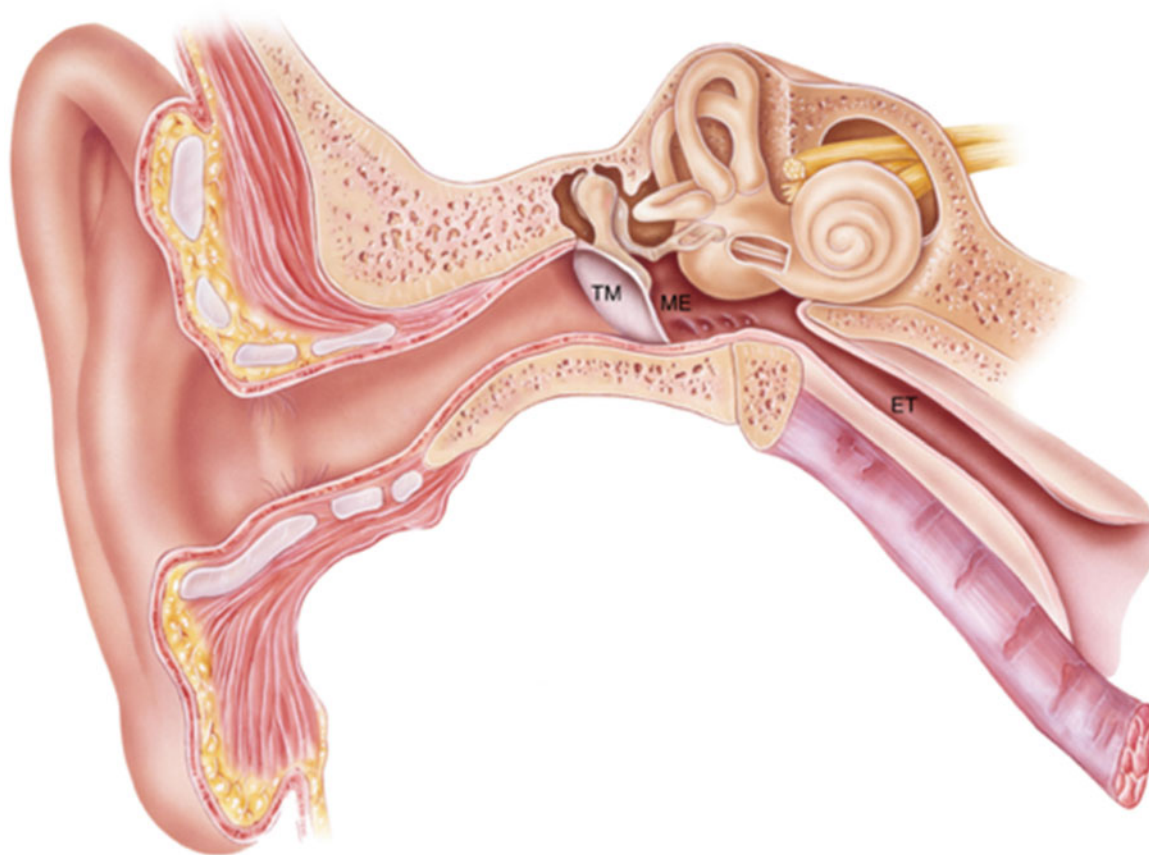


Fig. 1. The human ear. OM occurs within the middle ear (ME) cavity; the space behind the tympanic membrane (TM) which communicates with the post-nasal space via the Eustachian tube (ET).

OME is initiated by acute infection and then maintained by non-infective host factors, or whether failure to fully eradicate an infective nidus leads to persistent inflammation.

In favour of the former, there are mechanisms which can lead to persistent 'sterile' inflammation in the middle ear. Debris from damaged cells, for example, remnants of bacterial cell walls (Refs 21, 22), can trigger an inflammatory response via so-called danger-associated molecular patterns (DAMPs), which may contribute to COM subtypes, in the absence of clinical evidence of active infection.

In favour of the persistent infective stimulation theory, bacterial biofilms have been detected in both the middle ear (Ref. 23) and on adenoids (Ref. 24) (nasopharyngeal lymphoid tissue closely associated with the Eustachian tube orifice) of children with chronic middle ear disease more frequently than those without. These biofilms are extracellular matrices which house bacterial colonies and confer antimicrobial resistance (Ref. 25). It is plausible that such a system enables bacteria to escape host immune-mediated destruction and lead to persistent inflammation. However, even in effusions which do not have evidence of biofilm formation, live bacteria are frequently found (Ref. 26).

Whichever the stimulus, both DAMP and PAMP signalling pathways converge on the activation of common pro-inflammatory pathways, such as NF- κ B and activator protein-1 (AP-1) or interferon regulatory factor 3 (IRF3) which act as transcription factors to initiate a pro-inflammatory and type 1 interferon response which are essential for effective bacterial and viral clearance (Ref. 27).

Chronic otitis media

Eustachian tube opening is recognised as important for appropriate ventilation of the middle ear and dysfunction of this process

has been implicated in chronic phenotypes of OM (Ref. 28) – possibly via increasing the risk of pathogen translocation into the middle ear. Further, it has been shown that intracellular pathogen recognition proteins have a demonstrated role in murine OM; for example, nucleotide-binding oligomerisation domain-like (NOD) receptors. Both NOD1 and NOD2-deficient mice show prolonged OM; specifically, NOD1-deficient mice demonstrate reduced macrophage recruitment and delayed inflammatory responses from neutrophils, and NOD2-deficient mice show reduced overall leukocyte influx and impaired bacterial clearance (Ref. 29). Evidence of NOD deficiency in humans causing OM is lacking but interestingly human samples of cholesteatoma, which is a sequela of chronic OM in which there is an abnormal accumulation of keratinizing squamous epithelium in the middle ear, show the upregulation of NOD2 (Ref. 30) – suggesting the role of PRRs in progression from OM to chronic complications may change over the course of disease.

Furthermore, the bacteria identified in COM and OME (Table 2) frequently differ from those found in AOM. Therefore, it seems likely that a complex interplay of both host immunity and extrinsic microbiological factors is involved in the development of COM, but this remains an area in need of further work.

Middle ear inflammation

Acute and recurrent acute otitis media

The host inflammatory response involves a diverse repertoire of effector cells and cytokines. Neutrophils, the most abundant immune effector cells in humans, are the primary cell type initially recruited to sites of infection. They are attracted by

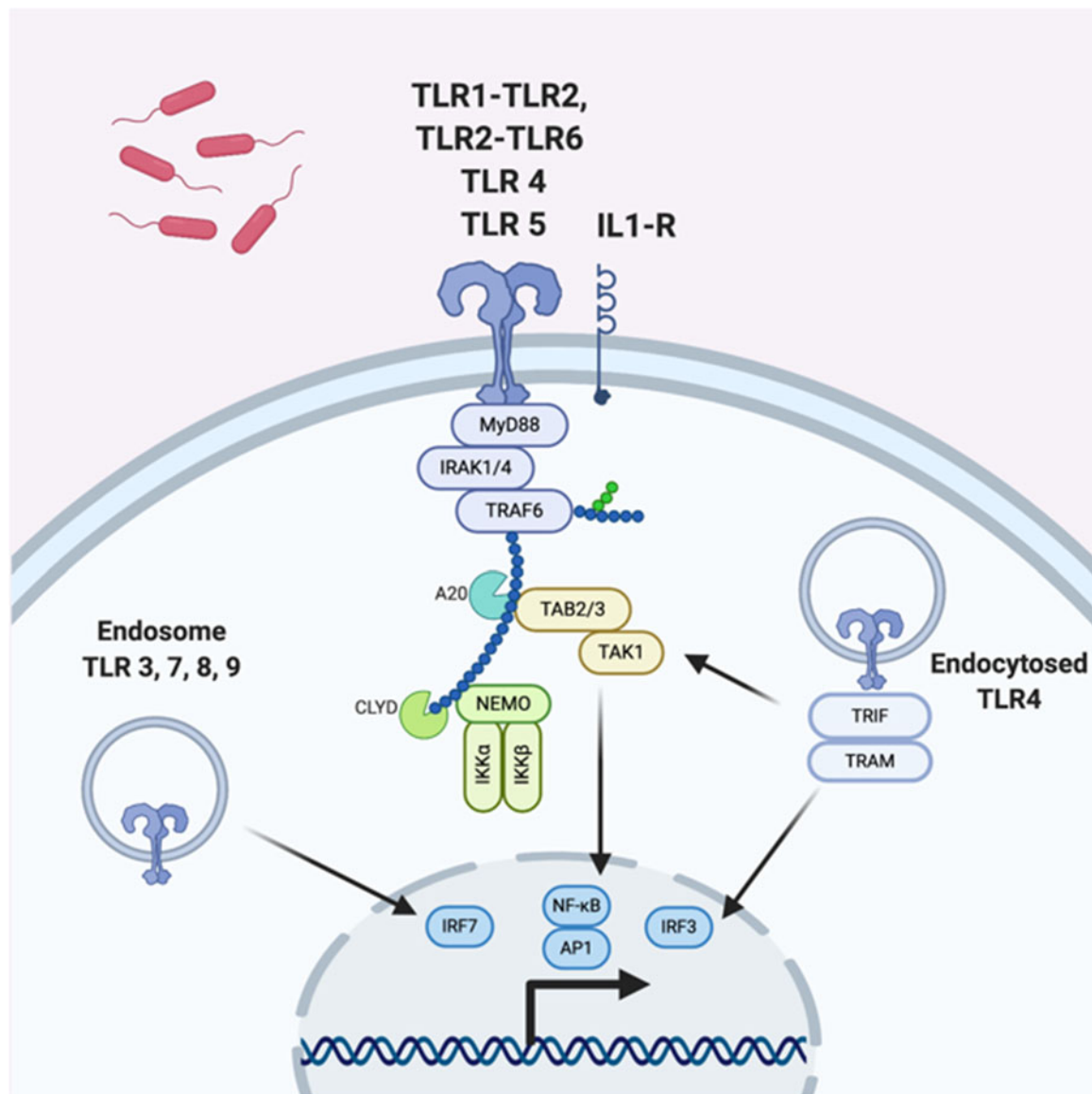


Fig. 2. TLR signalling mechanisms; mouse and human. Extracellular pathogens are detected by one of a range of TLRs which trigger downstream signalling via adaptor proteins including MyD88 and IRAK1/4. This leads to the upregulation of genes involved in generating a pro-inflammatory response. Adapted from O'Neill *et al.* (Ref. 10).

chemotactic stimuli, such as CXCL8 (IL-8), which is secreted by tissue resident macrophages or indeed infected tissue itself. Consequently, IL-8 in middle ear effusion is significantly more elevated in culture-positive acute OM than culture-negative OM (Ref. 32), and is higher in the effusion of acute OM-prone children than those who are not acute OM-prone (Refs 33, 34). IL-8 is also consistently higher in acute OM and COM than OME, and is associated with higher neutrophil counts in middle ear effusion (Ref. 35). Therefore, a means to modulate IL-8 signalling may be beneficial and would be a valuable area of further research – particularly in the context of those who are otitis prone.

Epithelial cells have important interactions with immune cells and consequently modulate airway inflammation. For example, healthy airway epithelium is a potent inhibitor of dendritic cell-induced T-cell activation (Ref. 36). Furthermore, epithelial cell damage, such as by viral infection, leads to loss of this T-cell inhibition and consequent airway inflammation (Refs 37, 38). It logically proceeds that epithelial damage in the middle ear cavity – for example, an episode of viral or bacterial acute OM – could impair T-cell regulation and facilitate tissue

inflammation. It may also be that an initial viral infection leads to ciliopathy which predisposes to secondary bacterial infection as occurs elsewhere in the respiratory tree (Ref. 39).

Insight from other upper airway mucosal sites may also be informative in understanding epithelial inflammation in OM. In chronic rhinosinusitis, for example, there is now emerging evidence of distinct endotypes which are each mediated by particular innate lymphoid cell (ILC1/2/3) and T helper cell (Th1/Th2/Th17) subgroups. It logically follows that treatments for these subgroups may differ – particularly with the advent of targeted monoclonal antibody or 'biologic' therapies (Ref. 40). Analogous molecular subgrouping in OM is lacking, but similar mechanisms seem plausible and may offer new routes to more personalised treatments.

Whilst they do not yet inform OM subgroups, the differential function of T-cell subgroups in OM has already been studied systemically; even from birth, enhanced T helper 1 and 2 cytokine production has been observed to be associated with a subsequently lower risk of middle ear infections in infancy (Ref. 41). Conversely, T cells from adenoids of children with recurrent acute OM appear to be more readily stimulated by bacteria and

Table 2. Bacterial species identified in COM and OME ranked in order of prevalence; adapted from (Ref. 31) and (Ref. 26)

COM	OME
<i>Pseudomonas aeruginosa</i>	Coagulase negative staphylococci
<i>Staphylococcus aureus</i>	<i>Veillonella</i> spp.
<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>
<i>Proteus mirabilis</i>	<i>Streptococcus pneumoniae</i>
<i>Proteus vulgaris</i>	<i>Bacillus</i> spp.
<i>Escherichia coli</i>	<i>Moraxella catarrhalis</i>
<i>Streptococcus pneumoniae</i>	<i>Pseudomonas</i> spp.

are more productive of IFN- γ than those from children without OM (Refs 42, 43). Therefore, there appears to be a dichotomy between whether elevated T-cell activity is protective against, or is a risk factor for, recurrent acute OM. It may be that the relevant abundances of specific subgroups of T cells are important. Indeed, there are suggestions that deficits in T-cell memory specifically contribute (Ref. 44). It may also be that there are divergences between systemic and tissue-resident T cells, and this would be an interesting avenue for future research.

The complement system, a protein constituent of serum and tissue fluid responsible for opsonisation and killing of bacteria, is also involved in governing the recruitment of immune effector cells in the middle ear. Knockout studies in mice have demonstrated increased susceptibility to *Streptococcus pneumoniae*-induced OM with impairment of either the classical or alternative complement pathways (Ref. 45). Indeed, *S. pneumoniae* strains able to restrict complement binding were more likely to cause invasive disease in a chinchilla model of OM (Ref. 46). Polymorphisms in mannose-binding lectin, which is responsible for complement-mediated pathogen surface recognition, have been identified as risk factors for recurrent acute OM; however, these seem to be mainly in younger age children (i.e. <2 years) when maternally-derived antibodies are no longer effective, but when adaptive immunity is not yet mature (Refs 47, 48, 49).

In humans, much of the localised inflammatory signalling triggered by immune infiltration is because of the production of cytokines. Although viruses are known to cause acute OM, their capacity to induce such pro-inflammatory mediators detectable in middle ear effusion appears to be limited compared with bacterial otopathogens (Ref. 50). In acute bacterial OM, there is vigorous production of proinflammatory cytokines including IL1 β , TNF and IL8, which rapidly decrease once bacteria are fragmented (Ref. 51). Defective production of such cytokines appears to confer an acute otitis-prone phenotype; particularly with polymorphisms in *TNFA*, *IL6* and *IL10* (Refs 52, 53). This suggests that a robust proinflammatory response is essential for bacterial clearance and defects in this process enable recurrent acute infections. This is supported by murine knock-out models affecting TNF signalling pathways, for example, *Tgfr^{-/-}* (Ref. 54), *Fbxo11^{-/-}* (Ref. 55) and *Evi1^{-/-}* (Ref. 56) which all demonstrate increased OM susceptibility. Interestingly, whilst TNF-deficient mice have defects in downstream protein production, for example, CCL3, the addition of recombinant TNF only partially restores macrophage phagocytic capacity but recombinant CCL3 completely reverses the impairment (Ref. 57). This is consistent with further work that shows *Ccl3^{-/-}* mice with induced OM have compromised bacterial clearance and thickening of middle ear mucosa, along with infection by atypical otopathogens, such as *Klebsiella pneumoniae*, increased bacterial load in the nasopharynx, and disrupted nasopharyngeal microbiome (Refs 58, 59).

This suggests that whilst TNF has pleiotropic effects on innate immune response, targeting particular downstream mediators may be more useful for therapeutic manipulation.

Otitis media with effusion

It remains uncertain whether OME is invariably a sequela of AOM which fails to fully resolve, or whether there are distinct instigator mechanisms at the beginning of episodes which subsequently manifest an OME phenotype. However, it is clear that complex multi-factorial defects in host immunity play a role in the development of OME. For example, macrophages extracted from chronic effusions have been shown to suppress the activities of lymphocytes; making them permissive to chronic infection (Ref. 60). Additionally, middle ear mucosal samples from children with chronic effusion have demonstrated intense complement activation (Ref. 61). It has also been observed that there are higher serum levels of some interleukins (e.g. IL-5) in children with OME than controls (Ref. 62) but, perhaps surprisingly, differences in serum IgG levels do not seem to play a role (Ref. 47). There are suggestions that the development of chronic mucosal disease might be a function of duration of inflammation, rather than the intensity of cytokine production (Ref. 63). Indeed, duration of inflammatory signalling may be relatively prolonged in the context of dense nasopharyngeal colonisation; which is in keeping with known risk factors for OME (Ref. 64).

The mucosal surfaces of the upper respiratory tract and middle ear themselves have evolved multiple ways to protect the host from infection and one key mechanism is the production of mucins. These large glycoproteins provide a key barrier function and are responsive to a range of inflammatory cytokines (Ref. 65). It has been observed that the presence of otopathogens triggers the upregulation of gel-forming mucins and indeed that polymicrobial infection can act synergistically to increase this further (Ref. 66). This upregulation of mucin genes in the middle ear, such as *MUC2*, *MUC5AC* and *MUC5B*, contributes to effusion viscosity (Ref. 67). *MUC5B*, in particular, seems to be especially high in mucoid as compared with serous effusions (Refs 68, 69). Interestingly, *MUC5B^{-/-}* mice show chronic middle ear infection and persistent inflammation (Ref. 70). So, whilst overproduction of some mucins is associated with viscous effusion formation which impairs the transmission of sounds through the middle ear and results in a conductive hearing loss, others likely play a critical role in capturing and removing pathogens. Indeed, at other upper airway sites, it has been identified that mucins may also lead to immune cell dysfunction and bacterial growth (Ref. 71).

Another key mechanism of mucosal defence is the barrier and biomechanical function of epithelial cells. The middle ear epithelium, like that of the lower airways, has a tight network of intercellular apical junctional complexes which regulate the paracellular secretion of antimicrobial peptides, in addition to preventing the ingress of undesired pathogens, allergens and particulate matter in order to avoid inappropriate tissue inflammation (Ref. 72). As OM has clinical correlations with infection, allergy and environmental contamination (Ref. 1), it seems likely that the barrier function of middle ear epithelium is likely critical in regulating tissue homeostasis.

However, it remains to be fully elucidated how the fluid effusions are actually generated in OME. Some studies have suggested that complement anaphylatoxins (such as C3a and C5a), which are responsible for chemoattraction of inflammatory cells, are significantly more elevated in middle ear effusion in persistent OM but whether this is cause or effect remains unclear (Ref. 73). Other work has identified that protein derivatives from neutrophils known as neutrophil extracellular traps (NETs), widely described

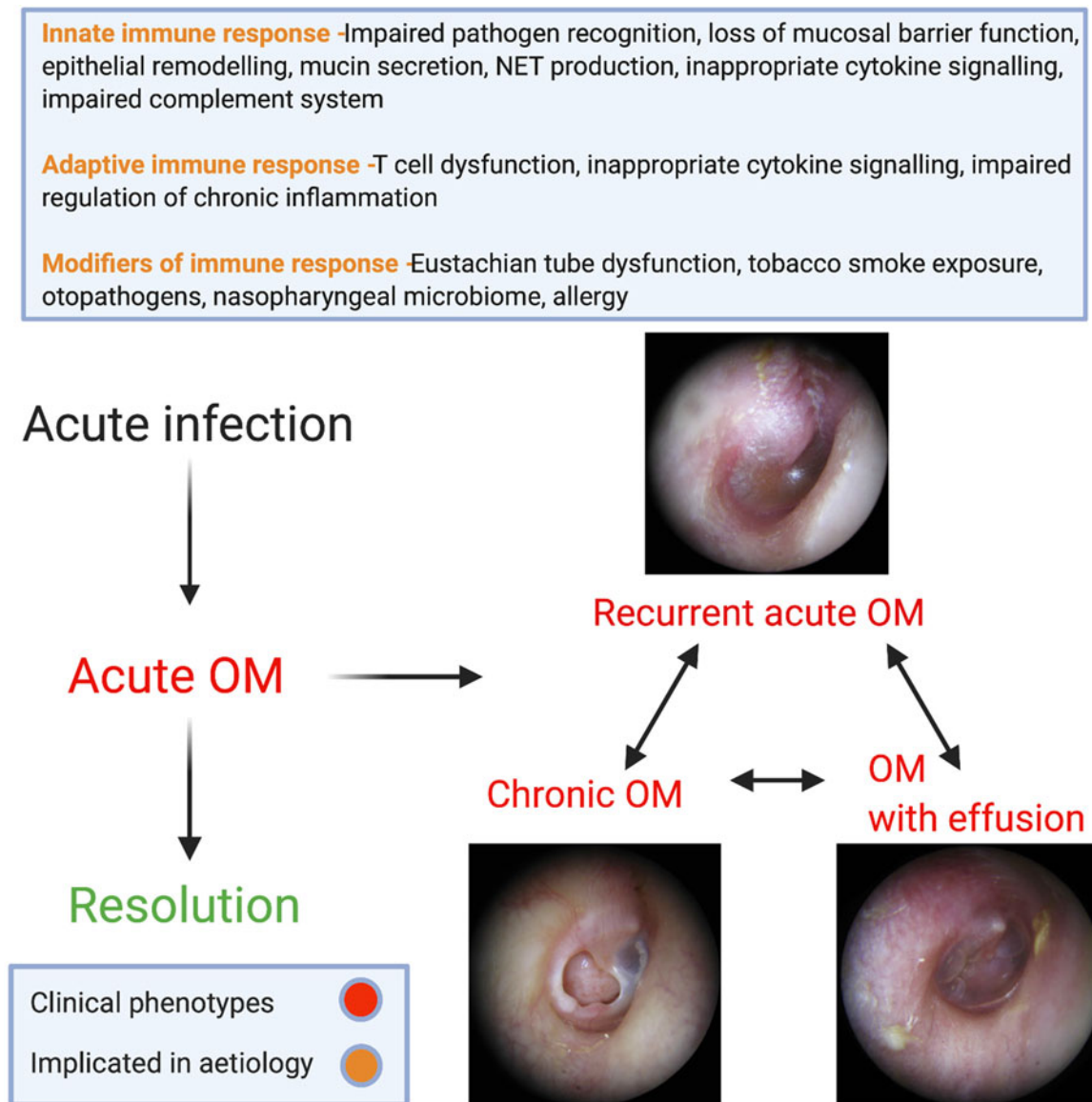


Fig. 3. Summary of implicated mechanisms of disease in subgroups of OM.

as an innate defence mechanism in pulmonary infections, surprisingly appear to facilitate secondary pneumococcal infection in the middle ear (Ref. 74). They have also been identified in middle ear effusion and appear to co-localise with MUC5B (Ref. 75) – an observation consistent with reports that NETs promote and sustain epithelial mucin secretion (Ref. 76), suggesting that NETosis may contribute to fluid viscosity in OME, even once the acute infection has resolved. Disruption of NETs may therefore be a valuable direction for resolving viscous middle ear effusions.

Alternative hypotheses can be derived from the observation that OME is more prevalent in children with concomitant allergic rhinitis than those without (Refs 77, 78), suggesting that congestion of the nasal mucosa leads to Eustachian tube dysfunction and negative middle ear pressure with consequent transudation of fluid. Allergy is also associated with an excessive Th2 response, driving high IL4 production – which is inhibitory to a Th1 (i.e. antibacterial) response, thereby permitting chronic infection and inflammation. Other observations in OME include the upregulation of hypoxia-related genes leading to increased angiogenesis (Ref. 79) and enzyme-mediated tissue damage (Ref. 80), but the relationship of these to the immune mechanisms above remain unclear.

Chronic otitis media

As with pathogen detection, the mechanisms governing mucosal inflammation in COM are less well understood than in AOM and OME. It is clear that the host middle ear epithelium is crucial for tissue homeostasis, the regulation of inflammation and antimicrobial defence, and is therefore likely of importance (Ref. 81). It is, however, notable that the middle ear epithelium is not homogenous in structure, and is therefore unlikely homogeneous in function. Recent work has described how mucosa morphology differs throughout the middle ear cavity from pseudostratified ciliated epithelium, with its functional capacity for bacterial eradication via mucociliary clearance around the Eustachian tube orifice, through to progressively thinner gas exchanging epithelium located more superiorly (Ref. 82). Transformation of this epithelium through partial epithelial to mesenchymal transition (EMT), that is, loss of cell-cell adhesion and apico-basal polarity in favour of mesenchymal traits of migration, invasion and producing extracellular matrix components, has been noted in samples from human cholesteatoma (Ref. 83). EMT has a clear role in the development of chronic lower airways diseases, such as chronic obstructive pulmonary disease and pulmonary fibrosis (Ref. 84); so it is plausible that

such a mechanism is at work in chronic middle ear inflammation. This is also consistent with observations that known risk factors for OM, such as smoking, are known to drive EMT (Ref. 85). However, as not all classical features of EMT are observed in cholesteatoma samples (e.g. tight junction formation and terminal differentiation are not affected), it may be that this represents a distinct pathological process compared with that observed in the other EMT-related biological processes, such as normal development, cancers and chronic lung disease (Ref. 86).

Persistent infection/inflammation can lead to tissue remodeling in the middle ear; for example, mucous glands become more prevalent in COM (Ref. 87). Paradoxically, over time, these mucous glands can degenerate; diminishing the capacity for mucin production, which may have important antimicrobial properties (Ref. 88). Indeed, it has been shown that the stroma in COM is infiltrated with macrophages and there is significantly lower expression of *Tlr2*, 4 and 5, but the upregulation of *TNF- α* , *IL-1 β* , *IFN- γ* and *IL-6* compared with healthy ears. Together these indicate a pro-inflammatory milieu which, over time, leads to histological and functional changes which diminish the host's capacity to respond to infection in those with COM (Ref. 89). There remains a question about whether these observations are driven by a dysfunctional host immune response or whether persistent infection is directly causing these changes. This would be a valuable area for future research.

Anti-inflammatory resolution in the middle ear

The dampening of inflammation in disease resolution has known importance at many anatomical subsites but an understanding of this in the middle ear is presently lacking. Generalised mechanisms include the production of anti-inflammatory mediators, abolition of neutrophil recruitment, neutrophil apoptosis, chemokine depletion and alternative activation of macrophages (Ref. 90). It seems likely that similar mechanisms are at work in the healthy middle ear, but experimental confirmation is awaited. It is known that anti-inflammatory cytokine genes, such as *IL10*, are upregulated on induction of acute OM, particularly with episodes secondary to *S. pneumoniae* (Ref. 91). Specific analogues of such pathways in AOM remain to be trialled and inhibitors of pro-inflammatory cytokines, including *TNF- α* , have only been trialled in animal models (Ref. 92).

Multiple medical treatments to resolve chronic inflammation in OME have been tested but the translational potential to date has been limited. For example, whilst steroids showed promise in reducing inflammation in a murine model of OM (Ref. 93), the OSTRICH trial; a double-blinded randomised trial comparing a short course of oral steroids with placebo in children with OME, did not demonstrate significant clinical benefit (Ref. 94). However, one in 14 children did have an improvement in hearing thresholds, suggesting a possible useful mechanistic element to suppression of inflammation in the middle ear.

Other therapeutic agents aimed at reducing middle ear inflammation have been investigated in animal models and cell lines. For example, Polymyxin B which binds to and inactivates bacterial endotoxin (found on the surface of Gram-negative bacteria) has shown promise in reducing cellular infiltrate, effusion volume and mucosal oedema in a guinea pig model of OME (Ref. 95), as has the manipulation of pathways which regulate mucosal inflammation, such as stimulation of the peroxisome proliferator-activated receptor-gamma (PPAR γ) pathway with agonist Ciglitazone which dampens the inflammatory response in the human middle ear epithelial cell (HMEECs) line in vitro (Ref. 96). Similarly, blockade of granulocyte/macrophage colony-stimulating factor – a potent proinflammatory peptide – results in reduced infiltration of inflammatory cells in middle

ear mucosa in a murine model of OM (Ref. 97). Metformin, most commonly used in the treatment of diabetes mellitus, has also been tested in vitro because of the reports of anti-inflammatory effects. Indeed in HMEECs treated with LPS, metformin suppressed the production of *TNF- α* , *COX-2* and intracellular reactive oxygen species (Ref. 98). Another agent, vinpocetine, which suppresses inflammation induced by *S. pneumoniae* through inhibition of ERK (Ref. 99) reduces middle ear inflammation in a murine model of OM. To date, none of these have been trialled in human OM.

A summary of the discussed mechanism of disease in OM subgroups is demonstrated in Figure 3.

Conclusion and future work

OM comprises a broad spectrum of interrelated pathologies. Defects in host immunity appear to play a key role in the response to initial infection and a complex range of modifying factors determine if, and which, of the subgroups subsequently emerges. At present, the limited translational literature does not reflect the substantial clinical burden of OM. Much information to date has been derived from cell lines and animal models of OM, which may not fully reflect the human pathology. Difficulties in the acquisition of human middle ear tissue have likely played a part in this and the development of physiological systems to study OM is required. If defects in mechanisms of disease could be identified, pharmacological manipulation would be an appealing alternative to existing surgical treatments; reducing both risk and cost to patients who, until now, find themselves with limited treatment options.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/erm.2021.10>

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Ethical standards. This review article required no specific approval from a research ethics committee.

References

- Schilder AGM *et al.* (2016) Otitis media. *Nature Reviews Disease Primers*. [cited 2018 Nov 14] 2, 16063. Available at <http://www.ncbi.nlm.nih.gov/pubmed/27604644>.
- Teele DW, Klein JO and Rosner B (1989) Epidemiology of otitis media during the first seven years of life in children in Greater Boston: a prospective, cohort study. *Journal of Infectious Diseases*. [cited 2018 Nov 1] 160:83–94. Available at <https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/160.1.83>.
- Bennett KE *et al.* (2001) Behaviour and developmental effects of otitis media with effusion into the teens. *Archives of Disease in Childhood* 85, 91–95.
- Liu T *et al.* (2017) NF- κ B signaling in inflammation. *Signal Transduction and Targeted Therapy*. [cited 2020 Nov 24] 2, 17023. Available at <http://www.nature.com/articles/sigtrans201723>.
- Short KR *et al.* (2013) Influenza-induced inflammation drives pneumococcal otitis media. *Infection and Immunity* 81, 645–652. Available at <http://iai.asm.org/content/81/3/645.full.pdf>.
- Mather MW *et al.* (2019) A systematic review and meta-analysis of antimicrobial resistance in paediatric acute otitis media. *International Journal of Pediatric Otorhinolaryngology*. [cited 2020 Nov 11] 123, 102–109. Available at <http://www.ncbi.nlm.nih.gov/pubmed/31085462>.

7. **Xu Q *et al.*** (2019) Comparative analysis of microbiome in nasopharynx and middle ear in young children with acute otitis media. *Frontiers in Genetics*. [cited 2020 Nov 11] **10**, 1176. Available at <http://www.ncbi.nlm.nih.gov/pubmed/31803245>.
8. **Teschner M** (2016) Evidence and evidence gaps in the treatment of Eustachian tube dysfunction and otitis media. *GMS Current Topics in Otorhinolaryngology – Head and Neck Surgery*. [cited 2021 Apr 15] **15**, Doc05. Available at <http://pmc/articles/PMC5169078/>.
9. **Abramson JS, Giebink GS and Quie PG** (1982) Influenza A virus-induced polymorphonuclear leukocyte dysfunction in the pathogenesis of experimental pneumococcal otitis media. *Infection and Immunity* **36**, 289–296.
10. **Kawasaki T and Kawai T** (2014) Toll-like receptor signaling pathways. *Frontiers in Immunology* **5**, 461. Available at <https://www.frontiersin.org/article/10.3389/fimmu.2014.00461>.
11. **Leichtle A *et al.*** (2012) The role of DNA sensing and innate immune receptor TLR9 in otitis media. *Innate Immunity* **18**, 3–13. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed13&NEWS=N&AN=364111751>.
12. **Leichtle A *et al.*** (2009) TLR4-mediated induction of TLR2 signaling is critical in the pathogenesis and resolution of otitis media. *Innate Immunity* **15**, 205–215. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed11&NEWS=N&AN=355018149>.
13. **Zhao SQ *et al.*** (2009) Role of interleukin-10 and transforming growth factor beta 1 in otitis media with effusion. *Chinese Medical Journal* **122**, 2149–2154. Available at <http://www.cmj.org/Periodical/PDF/200991559398140.pdf>.
14. **Huang Y *et al.*** (2016) TLR2 promotes macrophage recruitment and *Streptococcus pneumoniae* clearance during mouse otitis media. *Pediatric Research* **80**, 886–893. Available at <http://www.nature.com/pr/index.html>.
15. **Han F *et al.*** (2009) Role for toll-like receptor 2 in the immune response to *Streptococcus pneumoniae* infection in mouse otitis media. *Infection and Immunity* **77**, 3100–3108.
16. **Hafren L *et al.*** (2015) Predisposition to childhood otitis media and genetic polymorphisms within the toll-like receptor 4 (TLR4) locus. Yao Y-G, editor. *PLoS ONE*. [cited 2020 Nov 11] **10**, e0132551. Available at <https://dx.plos.org/10.1371/journal.pone.0132551>.
17. **Hernandez M *et al.*** (2008) Myeloid differentiation primary response gene 88 is required for the resolution of otitis media. *Journal of Infectious Diseases*. [cited 2020 Nov 11] **198**, 1862–1869. Available at <http://www.ncbi.nlm.nih.gov/pubmed/18986247>.
18. **Leichtle A *et al.*** (2009) The Toll-like receptor adaptor TRIF contributes to otitis media pathogenesis and recovery. *BMC Immunology* **10**, 45. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed11&NEWS=N&AN=355219940>.
19. **Picard C *et al.*** (2010) Clinical features and outcome of patients with IRAK-4 and MyD88 deficiency. *Medicine (Baltimore)*. [cited 2020 Nov 24] **89**, 403–425. Available at <http://www.ncbi.nlm.nih.gov/pubmed/21057262>.
20. **Schaar V *et al.*** (2011) Multicomponent *Moraxella catarrhalis* outer membrane vesicles induce an inflammatory response and are internalized by human epithelial cells. *Cellular Microbiology* **13**, 432–449. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed12&NEWS=N&AN=361253727>.
21. **Komori M *et al.*** (2011) Pneumococcal peptidoglycan-polysaccharides regulate toll-like receptor 2 in the mouse middle ear epithelial cells. *Pediatric Research*. [cited 2020 Nov 11] **69**, 101–105. Available at <http://www.nature.com/doi/10.1203/PDR.0b013e3182055237>.
22. **Bernstein JM, Praino MD and Neter E** (1980) Detection of endotoxin in ear specimens from patients with chronic otitis media by means of the limulus amoebocyte lysate test. *Canadian Journal of Microbiology* **26**, 546–548.
23. **Hall-Stoodley L *et al.*** (2006) Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. *Journal of the American Medical Association*. [cited 2020 Nov 11] **296**, 202–211. Available at <http://www.ncbi.nlm.nih.gov/pubmed/16835426>.
24. **Nistico L *et al.*** (2011) Adenoid reservoir for pathogenic biofilm bacteria. *Journal of Clinical Microbiology*. [cited 2021 Apr 15] **49**, 1411–1420. Available at <http://jcm.asm.org/>.
25. **Sharma D, Misba L and Khan AU** (2019) Antibiotics versus biofilm: an emerging battleground in microbial communities. *Antimicrobial Resistance and Infection Control*. BioMed Central Ltd. [cited 2021 Apr 15] **8**, 1–10. Available at <https://doi.org/10.1186/s13756-019-0533-3>.
26. **Daniel M *et al.*** (2012) Bacterial involvement in otitis media with effusion. *International Journal of Pediatric Otorhinolaryngology* **76**, 1416–1422.
27. **Liu T *et al.*** (2017) NF- κ B signaling in inflammation. *Signal Transduction and Targeted Therapy*. [cited 2020 Nov 11] **2**, 17023. Available at <http://www.nature.com/articles/sigtrans201723>.
28. **Fireman P** (1997) Otitis media and Eustachian tube dysfunction: connection to allergic rhinitis. *The Journal of Allergy and Clinical Immunology*. [cited 2020 Dec 9] **99**, S787–S797. Available at <http://www.ncbi.nlm.nih.gov/pubmed/9042072>.
29. **Lee J *et al.*** (2019) NOD1/NOD2-mediated recognition of non-typeable *Haemophilus influenzae* activates innate immunity during otitis media. *Innate Immunity* **25**, 503–512. Available at <http://www.sagepub.com/journals>.
30. **Leichtle A *et al.*** (2015) NOD-like receptor signaling in cholesteatoma. *BioMed Research International*. [cited 2020 Nov 11] **2015**, 1–9. Available at <http://www.hindawi.com/journals/bmri/2015/408169/>.
31. **Mittal R *et al.*** (2015) Current concepts in the pathogenesis and treatment of chronic suppurative otitis media. *Journal of Medical Microbiology*. Microbiology Society. [cited 2020 Dec 8] **64**, 1103–1116. Available at <https://pubmed.ncbi.nlm.nih.gov/26248613/>.
32. **Leibovitz E *et al.*** (2000) Interleukin 8 in middle ear fluid during acute otitis media: correlation with aetiology and bacterial eradication. *Archives of Disease in Childhood*. [cited 2020 Nov 11] **82**, 165–168. Available at <http://www.ncbi.nlm.nih.gov/pubmed/10648376>.
33. **Ghaheri BA *et al.*** (2007) Cochlear cytokine gene expression in murine acute otitis media. *Laryngoscope* **117**, 22–29. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed10&NEWS=N&AN=46066057>.
34. **Kaur R, Casey J and Pichichero M** (2016) Differences in innate immune response gene regulation in the middle ear of children who are otitis prone and in those not otitis prone. *American Journal of Rhinology & Allergy* **30**, e218–e223. Available at <http://docserver.ingentaconnect.com/deliver/connect/ocean/19458924/v30n6/s6.pdf?expires=1479726910&id=89236197&titleid=75004827&accname=Elsevier+BV&checksum=F770E758FA1EFF6C2F96381D2C68D09D>.
35. **Elmorsy S *et al.*** (2010) The role of IL-8 in different types of otitis media and bacteriological correlation. *The Journal of International Advanced Otolaryngology* **6**, 269–273.
36. **Ward C *et al.*** (2009) Bronchial epithelial cells cultured from clinically stable lung allograft patients promote the development of macrophages from monocytes rather than dendritic cells. *Thorax*. [cited 2020 Nov 6] **64**, 430–435. Available at <http://www.ncbi.nlm.nih.gov/pubmed/19158119>.
37. **Wang H, Su Z and Schwarze J** (2009) Healthy but not RSV-infected lung epithelial cells profoundly inhibit T cell activation. *Thorax*. [cited 2020 Nov 6] **64**, 283–290. Available at <http://www.ncbi.nlm.nih.gov/pubmed/18710906>.
38. **Brodlie M *et al.*** (2009) Airway epithelial cells as guardians of immune homeostasis? *Thorax*. [cited 2020 Nov 6] **64**, 1009. Available at <https://thorax.bmj.com/lookup/doi/10.1136/thx.2009.120121>.
39. **Kuek LE and Lee RJ** (2020) First contact: the role of respiratory cilia in host-pathogen interactions in the airways. *American Journal of Physiology-Lung Cellular and Molecular Physiology* **319**, L603–L619. Available at <https://doi.org/10.1152/ajplung.00283.2020>.
40. **Cao X-P *et al.*** (2018) Pathophysiologic mechanisms of chronic rhinosinusitis and their roles in emerging disease endotypes. *Annals of Allergy, Asthma & Immunology*. [cited 2021 Apr 16] **122**, 33–40. Available at <https://doi.org/10.1016/j.anai.2018.10.014>.
41. **Bergroth E *et al.*** (2017) Enhanced T helper 1 and 2 cytokine responses at birth associate with lower risk of middle ear infections in infancy. *Pediatric Allergy and Immunology* **28**, 53–59. Available at <http://www.wiley.com/bw/journal.asp?ref=0905-6157>.
42. **Kodama H *et al.*** (1999) Cellular immune response of adenoidal and tonsillar lymphocytes to the P6 outer membrane protein of non-typeable *Haemophilus influenzae* and its relation to otitis media. *Acta Oto-Laryngologica* **119**, 377–383. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed6&NEWS=N&AN=29227406>.
43. **Avanzini AM *et al.*** (2008) Children with recurrent otitis show defective IFN γ -producing cells in adenoids. *Pediatric Allergy and Immunology* **19**, 523–526. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed6&NEWS=N&AN=18266836>.

44. **Pichichero ME** (2020) Immunologic dysfunction contributes to the otitis prone condition. *Journal of Infection*. [cited 2020 Nov 24] **80**, 614–622. Available at <http://www.ncbi.nlm.nih.gov/pubmed/32205139>.
45. **Tong HH et al.** (2010) Enhanced susceptibility to acute pneumococcal otitis media in mice deficient in complement C1q, factor B, and factor B/C2. *Infection and Immunity* **78**, 976–983.
46. **Sabharwal V et al.** (2009) Role of complement in host defense against pneumococcal otitis media. *Infection and Immunity* **77**, 1121–1127.
47. **Straetmans M et al.** (2005) Immunological status in the aetiology of recurrent otitis media with effusion: serum immunoglobulin levels, functional mannose-binding lectin and Fc receptor polymorphisms for IgG. *Journal of Clinical Immunology*. [cited 2020 Nov 11] **25**, 78–86. Available at <http://link.springer.com/10.1007/s10875-005-0361-8>.
48. **Wiertsema SP et al.** (2006) Functional polymorphisms in the mannan-binding lectin 2 gene: effect on MBL levels and otitis media. *The Journal of Allergy and Clinical Immunology*. [cited 2020 Nov 11] **117**, 1344–1350. Available at <http://www.ncbi.nlm.nih.gov/pubmed/16750996>.
49. **Garred P et al.** (1993) Mannan-binding protein – levels in plasma and upper-airways secretions and frequency of genotypes in children with recurrence of otitis media. *Clinical and Experimental Immunology* **94**, 99–104.
50. **Stol K et al.** (2012) Inflammation in the middle ear of children with recurrent or chronic otitis media is associated with bacterial load. *Pediatric Infectious Disease Journal* **31**, 1128–1134. Available at <http://journals.lww.com/pidj>.
51. **Skovbjerg S et al.** (2010) High cytokine levels in perforated acute otitis media exudates containing live bacteria. *Clinical Microbiology and Infection* **16**, 1382–1388. Available at <https://www.journals.elsevier.com/clinical-microbiology-and-infection>.
52. **Emonts M et al.** (2007) Genetic polymorphisms in immunoresponse genes TNFA, IL6, IL10, and TLR4 are associated with recurrent acute otitis media. *Pediatrics* **120**, 814–823. Available at <http://pediatrics.aappublications.org/cgi/reprint/120/4/814>.
53. **Lindberg K, Rynnel-Dagoo B and Sundqvist K-G** (1994) Cytokines in nasopharyngeal secretions; evidence for defective IL-1beta production in children with recurrent episodes of acute otitis media. *Clinical and Experimental Immunology* **97**, 396–402.
54. **Tateossian H et al.** (2013) Otitis media in the Tgif knockout mouse implicates TGFβ signalling in chronic middle ear inflammatory disease. *Human Molecular Genetics*. [cited 2020 Nov 16] **22**, 2553–2565. Available at <https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/ddt103>.
55. **Hardisty-Hughes RE et al.** (2006) A mutation in the F-box gene, Fbxo11, causes otitis media in the Jeff mouse. *Human Molecular Genetics*. [cited 2020 Nov 11] **15**, 3273–3279. Available at <http://www.ncbi.nlm.nih.gov/pubmed/17035249>.
56. **Parkinson N et al.** (2006) Mutation at the Evil locus in Junbo mice causes susceptibility to otitis media. Beier D, editor. *PLoS Genetics*. [cited 2020 Nov 11] **2**, 1556–1564. Available at <https://dx.plos.org/10.1371/journal.pgen.0020149>.
57. **Leichtle A et al.** (2010) CC chemokine ligand 3 overcomes the bacteriocidal and phagocytic defect of macrophages and hastens recovery from experimental otitis media in TNF^{-/-} mice. *Journal of Immunology*. [cited 2018 Sep 6] **184**, 3087–3097. Available at <http://www.ncbi.nlm.nih.gov/pubmed/20164426>.
58. **Nuyen B, Deniffel D and Pak K** (2015) Absence of CCL3 compromises resolution of experimental otitis media and impairs host defense of the middle ear. *Journal of Investigative Medicine* **63**, 94. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed16&NEWS=N&AN=71770086>.
59. **Deniffel D et al.** (2017) Otitis media and nasopharyngeal colonization in ccl3^{-/-} mice. *Infection and Immunity* **85**, e00148–17. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med14&NEWS=N&AN=28847849>.
60. **Bernstein JM** (1985) Recent advances in otitis media with effusion. *Annals of Allergy, Asthma & Immunology*. [cited 2021 Apr 16] **55**, 544–551. Available at <https://europepmc.org/article/med/3901830>.
61. **Narkio-Makela M, Jero J and Meri S** (1999) Complement activation and expression of membrane regulators in the middle ear mucosa in otitis media with effusion. *Clinical and Experimental Immunology* **116**, 401–409.
62. **Johnston BNA et al.** (2008) Presence of otitis media with effusion and its risk factors affect serum cytokine profile in children. *International Journal of Pediatric Otorhinolaryngology*. [cited 2021 Apr 15] **72**, 209–214. Available at <https://pubmed.ncbi.nlm.nih.gov/18055023/>.
63. **Skotnicka B and Hassmann E** (2008) Proinflammatory and immunoregulatory cytokines in the middle ear effusions. *International Journal of Pediatric Otorhinolaryngology* **72**, 13–17. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed10&NEWS=N&AN=350198649>.
64. **Zhang Y et al.** (2014) Risk factors for chronic and recurrent otitis media—a meta-analysis. *PLoS ONE*. [cited 2020 Dec 9] **9**, e86397. Available at <http://www.ncbi.nlm.nih.gov/pubmed/24466073>.
65. **Linden SK et al.** (2008) Mucins in the mucosal barrier to infection. *Mucosal Immunology*. [cited 2020 Dec 9] **1**:183–197. Available at <http://www.nature.com/articles/mi20085>.
66. **Kerschner JE et al.** (2014) Differential response of gel-forming mucins to pathogenic middle ear bacteria. *International Journal of Pediatric Otorhinolaryngology*. [cited 2020 Nov 11] **78**, 1368–1373. Available at <http://www.ncbi.nlm.nih.gov/pubmed/24958163>.
67. **Mittal R et al.** (2014) Role of innate immunity in the pathogenesis of otitis media. *International Journal of Infectious Diseases*. [cited 2018 Nov 18] **29**, e259–e267. Available at <http://www.ncbi.nlm.nih.gov/pubmed/25447732>.
68. **Val S et al.** (2018) Characterization of mucoid and serous middle ear effusions from patients with chronic otitis media: implication of different biological mechanisms? *Pediatric Research*. [cited 2021 Apr 15] **84**, 296–305. Available at <https://pubmed.ncbi.nlm.nih.gov/3185811/>.
69. **Preciado D et al.** (2010) MUC5B is the predominant mucin glycoprotein in chronic otitis media fluid. *Pediatric Research* **68**, 231–236.
70. **Roy MG et al.** (2014) Muc5b is required for airway defence. *Nature*. [cited 2021 Jan 6] **505**, 412–416. Available at <https://pubmed.ncbi.nlm.nih.gov/24317696/>.
71. **Powell J et al.** (2018) Excess mucin impairs subglottic epithelial host defense in mechanically ventilated patients. *American Journal of Respiratory and Critical Care Medicine* **198**, 340–349. Available at <http://europepmc.org/abstract/med/29425465>.
72. **Ganesan S, Comstock AT and Sajjan US** (2013) Barrier function of airway tract epithelium. *Tissue Barriers*. [cited 2020 Nov 6] **1**, e24997. Available at <http://www.ncbi.nlm.nih.gov/pubmed/24665407>.
73. **He Y et al.** (2013) Complement activation in pediatric patients with recurrent acute otitis media. *International Journal of Pediatric Otorhinolaryngology*. [cited 2020 Nov 11] **77**, 911–917. Available at <http://www.ncbi.nlm.nih.gov/pubmed/23537925>.
74. **Short KR et al.** (2014) Antibodies mediate formation of neutrophil extracellular traps in the middle ear and facilitate secondary pneumococcal otitis media. *Infection and Immunity* **82**, 364–370. Available at <http://ia.asm.org/content/82/1/364.full.pdf>.
75. **Val S et al.** (2016) Proteomic characterization of middle ear fluid confirms neutrophil extracellular traps as a predominant innate immune response in chronic otitis media. *PLoS ONE*. [cited 2020 Nov 11] **11**, e0152865. Available at <http://www.ncbi.nlm.nih.gov/pubmed/27078692>.
76. **Zou Y et al.** (2018) Neutrophil extracellular traps promote lipopolysaccharide induced airway inflammation and mucus hypersecretion in mice. *Oncotarget*. [cited 2020 Nov 11] **9**, 13276–13286. Available at <http://www.ncbi.nlm.nih.gov/pubmed/29568356>.
77. **Pau BC and Ng DK** (2016) Prevalence of otitis media with effusion in children with allergic rhinitis, a cross sectional study. *International Journal of Pediatric Otorhinolaryngology* **84**, 156–160. Available at <http://www.elsevier.com/locate/ijporl>.
78. **Byeon H** (2019) The association between allergic rhinitis and otitis media: a national representative sample of in South Korean children. *Scientific Reports* **9**, 1610.
79. **Cheeseman MT et al.** (2011) HIF-VEGF pathways are critical for chronic otitis media in Junbo and Jeff mouse mutants. Avraham KB, editor. *PLoS Genetics*. [cited 2018 Nov 19] **7**, e1002336. Available at <https://dx.plos.org/10.1371/journal.pgen.1002336>.
80. **O'Reilly RC et al.** (2008) The role of extraesophageal reflux in otitis media in infants and children. *Laryngoscope*. [cited 2020 Nov 11] **118** (7 Part 2 Suppl 116), 1–9. Available at <http://doi.wiley.com/10.1097/MLG.0b013e31817924a3>.
81. **Gallo RL and Hooper LV** (2012) Epithelial antimicrobial defence of the skin and intestine. *Nature Reviews Immunology*. [cited 2020 Nov 24] **12**, 503–516. Available at <http://www.ncbi.nlm.nih.gov/pubmed/22728527>.
82. **Padurariu S et al.** (2019) On the functional compartmentalization of the normal middle ear. Morpho-histological modelling parameters of its

- mucosa. *Hearing Research*. [cited 2020 Nov 6] **378**, 176–184. Available at <https://www.sciencedirect.com/science/article/pii/S0378595518302338>.
83. **Takahashi M *et al.*** (2019) Partial epithelial–mesenchymal transition was observed under p63 expression in acquired middle ear cholesteatoma and congenital cholesteatoma. *Otology & Neurotology*. [cited 2020 Nov 6] **40**, E803–E811. Available at <http://www.ncbi.nlm.nih.gov/pubmed/31348131>.
 84. **Jolly MK *et al.*** (2018) Epithelial–mesenchymal transition, a spectrum of states: role in lung development, homeostasis, and disease. *Developmental Dynamics*. [cited 2020 Nov 6] **247**, 346–358. Available at <http://doi.wiley.com/10.1002/dvdy.24541>.
 85. **Milara J *et al.*** (2013) Epithelial to mesenchymal transition is increased in patients with COPD and induced by cigarette smoke. *Thorax*. [cited 2020 Dec 9] **68**, 410–420. Available at <http://www.ncbi.nlm.nih.gov/pubmed/23299965>.
 86. **Brabletz T *et al.*** (2018) EMT in cancer. *Nature Reviews Cancer*. Nature Publishing Group. [cited 2021 Apr 15] **18**, 128–134. Available at www.nature.com/nrc.
 87. **Bak-pedersen K and Tos M** (1973) Density of mucous glands in various chronic middle ear diseases. *Acta Oto-Laryngologica*. [cited 2020 Nov 11] **75**, 273–274. Available at <http://www.tandfonline.com/doi/full/10.3109/00016487309139710>.
 88. **Tos M and Caye-Thomassen P** (2002) Mucous glands in the middle ear – what is known and what is not. *ORL*. [cited 2020 Nov 11] **64**, 86–94. Available at <http://www.ncbi.nlm.nih.gov/pubmed/12021499>.
 89. **Si Y *et al.*** (2014) Attenuated TLRs in middle ear mucosa contributes to susceptibility of chronic suppurative otitis media. *Human Immunology* **75**, 771–776. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med8&NEWS=N&AN=24882571>.
 90. **Szondy Z *et al.*** (2017) Anti-inflammatory mechanisms triggered by apoptotic cells during their clearance. *Frontiers in Immunology* [cited 2020 Nov 11] **8**, 909. Available at <http://www.ncbi.nlm.nih.gov/pubmed/28824635>.
 91. **Liu K *et al.*** (2013) Higher Serum levels of interleukin 10 occur at onset of acute otitis media caused by *Streptococcus pneumoniae* compared to *Haemophilus influenzae* and *Moraxella catarrhalis*. *Laryngoscope* **123**, 1500–1505. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed14&NEWS=N&AN=52443550>.
 92. **Liu X, Sun J and Yin G** (2019) Expressions of MUC5AC and TNF-alpha in middle ear mucosa, and influence of anti-TNF-alpha McAb treatment on rat model of acute otitis media with effusion. *Tropical Journal of Pharmaceutical Research: TJPR* **18**, 1627–1631.
 93. **MacArthur CJ *et al.*** (2009) Steroid control of acute middle ear inflammation in a mouse model. *Archives of Otolaryngology – Head & Neck Surgery*. [cited 2018 Sep 4] **135**, 453–457. Available at <http://www.ncbi.nlm.nih.gov/pubmed/19451465>.
 94. **Francis NA *et al.*** (2018) Oral steroids for resolution of otitis media with effusion in children (OSTRICH): a double-blinded, placebo-controlled randomised trial. *The Lancet* **392**, 557–568. [cited 2018 Nov 18]. Available at www.thelancet.com.
 95. **Darrow DH and Keithley EM** (1996) Reduction of endotoxin-induced inflammation of the middle ear by polymyxin B. *Laryngoscope* **106**, 1028–1033. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed6&NEWS=N&AN=26287250>.
 96. **Jun HJ *et al.*** (2012) Ciglitazone inhibits cigarette smoke solution-induced inflammatory responses in human middle ear epithelial cells. *International Journal of Pediatric Otorhinolaryngology* **76**, 1136–1139. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed13&NEWS=N&AN=52017861>.
 97. **Kariya S *et al.*** (2013) Neutralizing antibody against granulocyte/macrophage colony-stimulating factor inhibits inflammatory response in experimental otitis media. *Laryngoscope* **123**, 1514–1518. Available at <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed14&NEWS=N&AN=52323208>.
 98. **Cho JG *et al.*** (2016) The suppressive effects of metformin on inflammatory response of otitis media model in human middle ear epithelial cells. *International Journal of Pediatric Otorhinolaryngology* **89**, 28–32. Available at <http://www.elsevier.com/locate/ijporl>.
 99. **Komatsu K *et al.*** (2020) Vinpocetine suppresses *Streptococcus pneumoniae* – induced inflammation via inhibition of ERK1 by CYLD. *Journal of Immunology* **204**, 933–942. Available at <https://www.jimmunol.org/content/jimmunol/204/4/933.full.pdf>.
 100. **Cochrane**. Chronic Otitis Media Cochrane Scoping Review. Available at https://ent.cochrane.org/sites/ent.cochrane.org/files/public/uploads/com_scoping_document_january_2017.pdf.