The Role of Immunity in the Development of Otitis Media

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Part I: The Role of Innate and Adaptive Immunity in Otitis Media

The term "immunity" comes from the Latin word *immunitas*, which refers to the protection from legal prosecution offered to Roman senators during their tenures in office. The immune system is responsible for protecting an organism against foreign substances, especially infectious microbes, and also products of damaged cells [1].

A normal immune response against microbes involves sequential and coordinated responses by different branches of the immune system (Table 8.1). Innate immunity is essential for the defense against microbes during the first few hours or days after infection, is mediated by mechanisms that are in place even before an infection occurs, facilitates rapid responses to the invading microbes, and stimulates adaptive immunity. Innate immunity detects microbial infections using pattern recognition receptors (PRRs) that are specific to molecules shared by groups of related microbes (pathogen-associated molecular patterns (PAMPs)). Adaptative immunity is stimulated by exposure to infectious agents and generates pathogen-specific immune responses, and it also has significant receptor diversity and memory. Immunological memory allows the adaptive system to increase in magnitude and defensive capabilities with each successive exposure to a particular agent.

Table 8.1 Components of innate and adaptive immunity

	Innate	Adaptive
Epithelial and chemical barriers	• Mucociliary apparatus • Mucous glycoproteins • Surfactants • Defensins, interferons, lactoferrin, and nitric oxide • Middle ear epithelial cells	Epithelial lymphocytes and antibodies
Blood proteins	Complement	Antibodies
Cells	• Neutrophils • Macrophages • Mast cells • Dendritic cells	• Lymphocytes T – CD4 Th1 Th2 Th17 – CD8 • Lymphocytes B

Innate Immunity

The innate system is composed of cellular and chemical barriers such as the skin, mucosal epithelia, antimicrobial peptides, blood proteins, including the complement system, and cells like macrophages and neutrophils.

Epithelial and Chemical Barriers

Mucosal immunity constitutes the first line of defense against respiratory pathogens in the respiratory tract. Epithelial cells of the middle ear contain several key defense mechanisms such as the mucociliary apparatus, the trapping function of mucous glycoproteins and surfactants, and the ability to secrete innate defense molecules such as defensins, interferons, lactoferrin, and nitric oxide [2].

Mucins are high-molecular-weight glycoproteins responsible for the viscous properties of middle ear effusion [3]. Although mucins are important components of innate immunity in the respiratory tract, they can also play a pathological role. Abnormally high levels of mucins have been demonstrated in middle ear effusions of chronic suppurative otitis media (OM) patients, preventing the transmission of sound waves and leading to conductive hearing loss. The upregulation of some mucin genes such as *MUC2*, *MUC5AC*, and *MUC5B* plays an important role in the pathogenesis of otitis media [2].

Surfactant proteins (SPs) such as SP-A are expressed in the middle ear and Eustachian tube and play an important role in innate responses through opsonization and comple-

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ment activation. SP-A opsonizes Gram-negative bacteria and modulates the expression of pro-inflammatory cytokines like interleukin (IL)-1β, IL-6, and tumor necrosis alpha (TNF-α). The immune function of SP-A in vivo has been studied using mouse models of otitis media, which demonstrated its role in enhancing bacterial phagocytosis and modulating middle ear inflammation [4].

Defensins are cationic proteins, whose main antimicrobial mechanisms are forming a pore in the microbial membrane and stimulating the production of pro-inflammatory cytokines and chemokines. Human B-defensin 2 and 3 are upregulated in the middle ear in response to bacteria and play a critical role in eliminating *Haemophilus influenzae* (*Hi*) [5, 6].

Middle ear epithelial cells express PRRs such as Toll-like receptors (TLRs) that detect infections by recognizing PAMPs and activate the innate immune response. Peptidoglycans such as those on the surface of *Haemophilus influenzae* (*Hi*) are recognized by TLR2; upon biding to its ligand, TLR2 initiates nuclear factor kappa B (NF-κB)-dependent cascades that activate the immune response and upregulate TLR2 expression in a positive feedback loop [7, 8]. Polymorphisms of the *TLR4* gene are associated with recurrent acute OM. When infected with *Hi*, TLR4 knockout mice had a worse mucosal immune response, with impairment of phagocytosis and phagosome maturation of polymorphonuclear cells as compared to wild-type mice [9]. Additional genes involved in the innate immunity have been found to be differentially regulated in acute otitis media (AOM)-prone children compared to healthy-age appropriate controls. Downregulation of TLR adaptor molecule 2 was found in middle ear fluid of 24 children with acute otitis media [10]. TLRs are not the only PRRs involved in the pathogenesis and recovery of otitis media; Nod-like receptors (NLRs) have also been shown to initiate and support robust immune responses through the production of inflammatory cytokines and recruitment of leukocytes to the middle ear [11].

Finally, impairment of epithelial and chemical barriers in otitis-prone children has been observed. They have lower capacity for epithelial repair, lower pro-inflammatory neutrophil chemoattractants such as macrophage inflammatory protein-1β (MIP-1β), IL-8, and CXCL5 [12], and pro-inflammatory cytokines of higher levels such as IL-2 and lower levels such as IL-7, IL-6, and IL-10 in nasal washes, thus showing that middle ear cytokine responses mirror those of the nasal mucosa versus the peripheral blood and suggesting that proximal mucosal sites may better predict the quality of middle ear responses compared to peripheral blood [13].

The Complement System

The complement system consists of several plasma proteins that work together to opsonize microbes, promote recruitment of phagocytes to the sites of infection, and, in some cases, directly kill the microbes [1]. There are three pathways of complement activation, among which the most important for responding to capsulated bacteria is the classical pathway, which is one of the major effector mechanisms of the humoral arm of adaptive immune responses.

Cells

Neutrophils are the most abundant leukocytes and the first line of defense against invading pathogens in the middle ear, experiencing roughly a 600-fold increase during acute otitis media [14]. They express multiple TLRs and play a crucial role in eradicating middle ear infections [2]. Upon activation, they form neutrophil extracellular traps (NETs) that are positively correlated with higher bacterial loads within middle ear fluids and surface-attached bacteria and contribute to effusion viscosity, thus leading to chronic suppurative otitis media [15].

Macrophages are also present in middle ear effusions, and their role in infection depends on the causative agent. *Streptococcus pneumoniae* serotypes 14 and 19F were found to be resistant to phagocytosis that can lead to bacterial antigens being trapped in the middle ear, thus promoting effusion [2]. *Haemophilus influenzae* utilizes a system of phase-variable epigenetic regulation, to facilitate adaptation and survival by evading opsonization, the process by which it is marked for destruction by macrophages [16].

Mast cells are distributed throughout the tubotympanum, predominantly in the pars flaccida, and can trigger allergic rhinitis, thus causing persistent inflammation that can lead to tube dysfunction and also impediment of mucociliary function that can lead to recurrent otitis media with effusion [17]. A possible role of mast cells and their cytokines in the pathogenesis of chronic serous otitis media has been suggested as these cells are increased in the patient's adenoid tissue and in thymic stromal lymphopoietin [18].

A normal tympanic membrane also contains abundant dendritic cells that have the potential to migrate and activate T cells. A significant increase in the number of these cells has been found in tubotympanic disease and in atticoantral disease, with the difference being more pronounced in the latter form of otitis media [19].

Adaptive Immune Responses

The adaptive immune system is composed of T and B lymphocytes and their products. There are two branches of adaptive immunity, namely, humoral immunity, which is mediated by antibodies and B cells, and cell-mediated or cellular immunity mediated by T cells.

Humoral Immunity

A humoral immune response combats microbes in many ways. Antibodies bind to microbes and prevent them from infecting cells, thus neutralizing the microbes. In fact, antibody-mediated neutralization is the only mechanism of adaptive immunity that stops an infection before it is established; this is why eliciting the production of potent antibodies is the key goal of vaccination. Immunoglobulin (Ig)G antibodies coat microbes and target them for phagocytosis because phagocytes (neutrophils and macrophages) express receptors for parts of IgG molecules. Both IgG and IgM activate the complement system, and complement products promote phagocytosis and destruction of microbes. IgA is secreted from mucosal epithelia and neutralizes microbes in the lumens of mucosal tissues, such as the respiratory and gastrointestinal tracts, thus preventing inhaled and ingested microbes from infecting the host [1].

There are differences between children and adult's humoral immunity, and the susceptibility of infants to AOM wanes with age due to immunological maturation. During pregnancy, IgG antibodies are passively transferred to the infant and progressively decrease during extrauterine life, reaching their lowest point at 6 months of life. The production of IgM and IgA begins progressively from birth. The capacity to respond to protein antigens is approximately 80% at birth and achieves levels like those of adults around 3 months of life. The ability to respond to polysaccharide antigens is not optimal until 2 years of life due to the absence of B cells in the marginal zone of the spleen [1].

For the normal development of humoral immunity, a correct development of B cells and a normal interaction of these with circulating T lymphocytes is necessary. Developing antibody-mediated immunity to *Streptococcus pneumoniae* and non-typeable *Hi* (NT*Hi*), the two most common pathogens causing AOM, is a cardinal step in preventing recurrent infections in young children. Upon receiving T cell help, B lymphocytes that recognized an antigen proliferate and differentiate into plasma cells that secrete different classes of antibodies with distinct functions. Polysaccharides and lipids stimulate secretion mainly of the antibody class called immunoglobulin M (IgM). Protein antigens induce the production of antibodies of different classes (IgG, IgA, IgE) from a single clone of B cells.

Otitis prone children have lower serum bactericidal antibody titers against pneumococcal proteins: histidine triad protein D (PhtD), pneumococcal choline binding protein A (PcpA) and pneumolysin (PlyD1) compared with nonotitis prone children after nasopharyngeal colonization and acute otitis media [20]. This may be due to poor memory B-cell and T-helper cell generation associated with reduced levels of pneumococcal-specific IgG in the serum after the infection [21].

Comparing acute to convalescent antibody titers after AOM, otitis-prone children had no significant change in total IgG responses to three *Hi* proteins (protein D, P6, and OMP26), whereas non-otitis-prone children had significant increases in protein D. Anti-protein D, P6, and OMP26 antibody levels measured longitudinally during *Hi* colonization between the ages of 6 and 24 months demonstrated subtle anti-protein D IgG increases over time in otitis-prone children compared to more than fourfold increases in non-otitis-prone children [22]. The raise in the antibody's levels in non-otitis-prone children probably prevents them from having recurrent otitis.

Cellular Immunity

T lymphocytes consist of two functionally distinct populations: helper T cells or $CD4^+$ cells and cytotoxic T lymphocytes (CTLs) or $CD8^+$ cells. The functions of helper T cells are mainly mediated by secreted cytokines, whereas CTLs produce molecules that directly kill other cells. $CD4^+$ T cells comprise functionally distinct populations characterized by specific transcription factors and cytokine profiles; T helper 1 (Th1), Th2, and Th17 [1].

Antigen-specific $CD4^+$ T cells have been shown to reduce *Streptococcus pneumoniae* nasopharyngeal colonization. An effective pathogen-specific T-cell response in adults has been associated with protection from invasive *Streptococcus pneumoniae* disease (invasive pneumococcal disease, IPD) and chronic obstructive pulmonary disease (COPD) caused by *Streptococcus pneumoniae* and NT*Hi*, respectively. More recently, Th17 cells have been described to mediate antibody-independent protection in a mouse model of pneumococcal infection. Moreover, $CD4^+$ T cells in samples collected from the adenoids and tonsils of traditionally defined otitis-prone children showed no proliferation in response to NT*Hi* protein P6, which led the authors to conclude that otitis-prone children lack pathogen-specific T cells. [23] Other authors have shown that adenoids have a reduced capacity to produce interferon-gamma (IFN-γ) and speculate that this alteration could cause susceptibility to recurrent acute otitis media [24].

For several decades, Pichichero et al. studied the underlying pathogenesis of AOM in children and also why the risk of AOM decreases over time. They observed that this susceptibility is not only due to a Eustachian tube dysfunction but also due to immune factors [13]. Peripheral blood mononuclear cells (PBMCs) from otitis-prone children between the ages of 6 and 12 months display a general skewing away from Th1 and Th17 immunity and toward Th2 and regulatory T cell (Treg) dominance [25]. This abnormality was largely outgrown by 3 years of age, coinciding with the epidemio-

logical observation of diminishing AOM at that age [13]. They also showed that otitis-prone children are more frequently diagnosed with viral upper respiratory infections possibly due to deficient antiviral responses at the nasopharynx with decreased production of pro-inflammatory cytokines and chemokines like IL-6, IL-10, and TNF-α [26].

Part II: The Ear Microbiota

The microbiota plays critical roles in the regulation and development of the major components of the host's immune system, whereas the immune system orchestrates the maintenance of the key features of the host–microbe symbiosis [27].

The human microbiota consists of ecological communities of commensal, symbiotic, and pathogenic microorganisms that colonize several body sites and play a critical role in the regulation of many homeostatic processes, including inflammation and defense against pathogens, to inhibit the colonization and growth of otopathogens [28]. Immediately after birth, the respiratory tract becomes colonized, and, in the first week of life, there is a predominance of *Staphylococcus* spp., *Corynebacterium*, *Dolosigranulum*, and *Moraxella*. This early bacterial colonization plays a pivotal role in the stability of microbial communities: profiles dominated by *Moraxella* and *Dolosigranulum*/*Corynebacterium* are associated with a stable microbiota and with lower rates of respiratory infections in later stages of life, whereas the less stable profiles are associated with a high abundance of *Hi* and *Streptococcus* [29].

Several environmental factors can influence the shaping of the microbiota's composition in the first years of life. Children born by vaginal delivery have predominance of bacteria previously associated with microbiome stability and respiratory health, but some authors suggest that this impact disappears at 6 weeks of age. Breastfed infants develop a bacterial profile enriched by *Dolosigranulum* and *Corynebacterium* at 6 weeks of age in comparison with formula-fed infants; however, this effect also disappears around 6 months of age. In children with AOM, recent antibiotic therapy induces a reduction of beneficial bacteria such as *Streptococcaceae* and *Corynebacteriaceae* and an increased abundance of *Enterobacteriaceae* and *Pasteurellaceae* in the upper respiratory tract. The effect of the conjugated pneumococcal vaccines in the microbiome is controversial and it seems to vary by ethnicity; Swiss vaccinated children have an increase in beneficial bacteria and in bacterial diversity, whereas in children from Gambia, vaccination reduced the nasopharyngeal carriage of vaccine serotypes, but pneumococcal carriage remained high among vaccinated infants, probably because of an immediate expansion of non-vaccine serotypes [30].

According to the pathogen reservoir hypothesis (PRH), the adenoid pad serves as a source of pathogens that can grow in this region and further spread to the respiratory system and middle ear, leading to infections and diseases [31]. Owing to the introduction of culture-independent techniques such as gene analysis with a polymerase chain reaction (PCR) using primers that target a segment of the *16SrRNA* gene, microbiological investigations now allow the knowledge of entire bacterial communities. There are keystone species that maintain the balance and function of the bacterial community such as *Dolosigranulum* spp. and *Corynebacterium* spp. In children, a diverse microbiota and a higher relative abundance of *Corynebacterium*, *Dolosigranulum*, *Propionibacterium*, *Lactococcus*, and *Staphylococcus* were associated with a lower incidence of *S. pneumoniae*, *H. influenzae*, and *Moraxella catarrhalis* colonization, lower AOM, a shorter course of AOM, and a better clinical outcome [30]. An unstable microbiota during an acute respiratory tract infection episode with the predominance of otopathogens is associated with the occurrence of a symptomatic viral infection and with a higher risk of transition to otitis, whereas children with asymptomatic viral infections had no predominance of otopathogens [32]. There are several trials of probiotic administration for prevention of middle ear diseases in children, but there is lack of evidence for their use [33].

References

1. Abbas A, Lichtman A, Pillai S. Cellular and molecular immunology, vol. 1. Elsevier; 2019. p. 1–2.
2. Mittai R, Kodiyan J, Gerring R, Mathee K, Li J-D, Grati M, et al. Role of innate immunity in the pathogenesis of otitis media. Int J Infect Dis. 2014;0:29–267.
3. Mittal R, Grati M, Gerring R, Blackwelder P, Yan D, Li JD, et al. In vitro interaction of Pseudomonas aeruginosa with human middle ear epithelial cells. PLoS One. 2014;9(3):1–11.
4. Abdel-Razek O, Ni L, Yang F, Wang G. Innate immunity of surfactant protein A in experimental otitis media. Innate Immun. 2019;25(7):391–400.
5. 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(1):24–38.
6. Lee HY, Takeshita T, Shimada J, Akopyan A, Woo JI, Pan H, et al. Induction of beta defensin 2 by NTHi requires TLR2 mediated MyD88 and IRAK-TRAF6-p38MAPK signaling pathway in human middle ear epithelial cells. BMC Infect Dis. 2008;8:87.
7. Chen R, Lim JH, Jono H, Gu XX, Kim YS, Basbaum CB, 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(3):1087–94.
8. Shuto T, Imasato A, Jono H, Sakai A, Xu H, Watanabe T, et al. Glucocorticoids synergistically enhance nontypeable Haemophilus

influenzae-induced toll-like receptor 2 expression via a negative cross-talk with p38 MAP kinase. J Biol Chem. 2002;277(19):17263–70. https://doi.org/10.1074/jbc.M112190200.
9. Hirano T, Kodama S, Fujita K, Maeda K, Suzuki M. Role of Toll-like receptor 4 in innate immune responses in a mouse model of acute otitis media. FEMS Immunol Med Microbiol. 2007;49(1):75–83.
10. Kaur R, Casey J, Pichichero M. Differences in innate immune response gene regulation in the middle ear of children who are otitis prone and in those not otitis prone. Am J Rhinol Allergy. 2016;30(6):e218–23.
11. Lee J, Leichtle A, Zuckerman E, Pak K, Spriggs M, Wasserman SI, et al. NOD1/NOD2-mediated recognition of non-typeable Haemophilus influenzae activates innate immunity during otitis media. Innate Immun. 2019;25(8):503–12.
12. Verhoeven D, Nesselbuch M, Pichichero M. Lower nasopharyngeal epithelial cell repair and diminished innate inflammation responses contribute to the onset of acute otitis media in otitis-prone children. Med Microbiol Immunol. 2013;202(4):295–302.
13. Pichichero M. Immunologic dysfunction contributes to the otitis prone condition. J Infect. 2020;80(6):614–22.
14. Morris MC, Pichichero ME. Streptococcus pneumoniae burden and nasopharyngeal inflammation during acute otitis media. Innate Immun. 2017;23(8):667–77.
15. Simon D, Simon HU, Yousefi S. Extracellular DNA traps in allergic, infectious, and autoimmune diseases. Allergy Eur J Allergy Clin Immunol. 2013;68(4):409–16.
16. Robledo-Avila FH, Ruiz-Rosado JD, Partida-Sanchez S, Brockman KL. A bacterial epigenetic switch in non-typeable Haemophilus influenzae modifies host immune response during otitis media. Front Cell Infect Microbiol. 2020;10(October):1–15.
17. Quaranta N, Iannuzzi L, Gelardi M. Does the type of rhinitis influence development of otitis media with effusion in children? Curr Allergy Asthma Rep. 2014;14(11):1–5.
18. Kumral TL, Dikker O, Yıldırım G, Karaketir S, Altındağ C, Çakın MC, et al. The role of thymic stromal lymphopoietin in the development of chronic otitis media with effusion. Eur Arch Otorhinolaryngol. 2021;279:1937. https://doi.org/10.1007/s00405-021-06995-z.
19. Jacob TM, Indrasingh I, Yadav BK, Rupa V. Langerhans cells in the human tympanic membrane in health and disease: a morphometric analysis. Otol Neurotol. 2013;34(2):325–30.
20. Xu Q, Casey JR, Newman E, Pichichero ME. Otitis-prone children have immunologic deficiencies in naturally acquired nasopharyngeal mucosal antibody response after streptococcus pneumoniae colonization. Pediatr Infect Dis J. 2016;35(1):54–60.
21. 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(8):1225–9.
22. Kaur R, Casey J, Pichichero M. Serum antibody response to three non-typeable Haemophilus influenzae outer membrane proteins during acute otitis media and nasopharyngeal colonization in otitis prone and non-otitis prone children. Vaccine. 2011;29(5):1023–8. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3624763/pdf/nihms412728.pdf.
23. Sharma S, Pichichero M. Cellular immune response in young children accounts for recurrent acute otitis media. Curr Allergy Asthma Rep. 2013;13(5):495.
24. Avanzini AM, Castellazzi AM, Marconi M, Valsecchi C, Marseglia A, Ciprandi G, et al. Children with recurrent otitis show defective IFNγ-producing cells in adenoids. Pediatr Allergy Immunol. 2008;19(6):523–6.
25. Surendran N, Nicolosi T, Kaur R, Pichichero ME. Peripheral blood antigen presenting cell responses in otitis-prone and non-otitis-prone infants. Innate Immun. 2016;22(1):63–71.
26. Ren D, Xu Q, Almudevar AL, Pichichero ME. Impaired pro-inflammatory response in stringently defined otitis-prone children during viral upper respiratory infections. Clin Infect Dis. 2019;68(9):1566–74.
27. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res. 2020;30(6):492–506. https://doi.org/10.1038/s41422-020-0332-7.
28. Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, et al. The NIH Human Microbiome Project. Genome Res. 2009;19(12):2317–23.
29. Paradise JL, Rockette HE, Colborn DK, Bernard BS, Smith CG, Kurs-Lasky M, et al. Otitis media in 2253 Pittsburgh-area infants: prevalence and risk factors during the first two years of life. Pediatrics. 1997;14(1):121–8.
30. Folino F, Ruggiero L, Capaccio P, Coro I, Aliberti S, Drago L, et al. Upper respiratory tract microbiome and otitis media intertalk: lessons from the literature. J Clin Med. 2020;9(9):1–27.
31. Nistico L, Kreft R, Gieseke A, Coticchia JM, Burrows A, Khampang P, et al. Adenoid reservoir for pathogenic biofilm bacteria. J Clin Microbiol. 2011;49(4):1411–20.
32. Lappan R, Imbrogno K, Sikazwe C, Anderson D, Mok D, Coates H, et al. A microbiome case-control study of recurrent acute otitis media identified potentially protective bacterial genera. BMC Microbiol. 2018;18(1):1–20.
33. Scott AM, Clark J, Julien B, Islam F, Roos K, Grimwood K, et al. Probiotics for preventing acute otitis media in children. Cochrane Database Syst Rev. 2019;2019(6):CD012941.