Immunologic Aspects of Otitis Media

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The middle ear cleft is a modified gas pocket which functions normally when the gas contents are regulated by a normal eustachian tube, resulting in equalization of middle ear pressure to that of the environment. The most important regulator of this middle ear pressure is the eustachian tube, a critical passageway from the nasopharynx into the middle ear. Any alteration of eustachian tube mucociliary function caused by virus, allergy, pollutants, or alteration of the normal homeostasis of the nasopharynx will result in eustachian tube obstruction. This, in turn, leads to underventilation of the middle ear, and transudation of fluid. If bacteria or virus or viral-bacterial interaction leads to infectious disease of the middle ear, an immune response is produced as a result of the inflammatory response, allowing lymphocytes and antigen-presenting cells to enter into the middle-ear mucosa. This article summarizes the immunologic reactivity in the middle ear following a viral-bacterial inflammatory reaction in the middle-ear mucosa. Although secretory IgA is critical for protection of the nasopharynx, its function in the middle ear has still not been resolved. The evidence strongly suggests that IgG1 and IgG3 subclasses are responsible for eradication of middle ear pathogens. Finally, a review of alternative approaches to the prevention of otitis media is briefly discussed in this critical period of emergence of resistant bacteria to available antibiotics.

Introduction

The middle ear space can be defined as a modified gas pocket that is connected to the nasopharynx via the eustachian tube. Like the alveolus of the lung, the middle ear cavity is maintained by the partial pressures of carbon dioxide (CO_2), oxygen (O_2), nitrogen (N_2), and water vapor [1]. The origin of these gases is mainly the diffusion from tissue lining the middle ear cleft and equalization of pressure from the nasopharynx via the eustachian tube into the middle ear cleft.

The middle ear space is normally sterile and possesses very few immunocompetent cells [2]. Therefore, the middle ear tissue and the middle ear cleft (eustachian tube, middle ear, and mastoid cavity) only develop an immune response after inflammation has occurred within this system. The stimuli that are involved in middle ear inflammation include viruses, bacteria, and most likely, inhalant allergens. The role of food allergens is still very controversial in the development of otitis media.

The events leading to inflammation of the middle ear often involve alterations in the normal micro-ecology of the nasopharyngeal bacterial flora, and a change in the nasopharyngeal mucosal immune response. This paper focuses on the microbiology and the immune response in the nasopharynx and the middle ear cleft in children with otitis media. The following subjects are briefly discussed: 1) the pathogenesis of otitis media; 2) lymphocyte trafficking into the middle ear required for the development of an immune response in otitis media; 3) the host defense mechanism against various bacteria that are associated with otitis media; and 4) some thoughts for the future regarding other strategies for protection against recurrent otitis media other than antibiotic therapy.

Pathogenesis of Otitis Media

The pathogenesis of otitis media requires invasion of the middle ear space by viruses, bacteria, or both. In the normal, healthy child, there is usually an efficient local immunologic mechanism in the nasopharynx, and a normal micro-ecology of nasopharyngeal flora, which are maintained in homeostasis in nasopharyngeal mucus [3]. The ratio of *viridans streptococci*, the most important and abundant normal commensal organism, to nontypable *Haemophilus influenzae* (NTHi), is somewhere between 5:1 to 7:1 [4]. Furthermore, the percentage of NTHi is probably less than 5% of the total flora. Also, in the normal nasopharynx, the middle ear pressure is similar to that of the barometric pressure of the atmosphere. There is a normal amount of mucus and surfactant in the eustachian tube, and the normal mucociliary system predominates. Finally, the mucosal immune system of the nasopharynx possesses secretory IgA. This, as well as some IgG subclasses created according to the genetic ability of that child to synthesize normal levels of secretory IgA and IgG subclasses, maintains this normal ratio of bacteria in the nasopharynx, wherein the commensal organisms are predominant, and the potential pathogens, although present, are downregulated by the presence of inhibitory *viridans streptococci*. The pathogenesis of otitis media, there-

fore, must involve some perturbation of the homeostasis of the nasopharynx and the nasopharyngeal flora. The two most common triggers for this alteration of normal homeostasis are a viral infection, or allergic rhinitis. A third possibility consists of pollution in the atmosphere such as cigarette smoke, sulfur dioxide, and any other types of environmental pollution. These pollutants may irritate the nasopharynx, altering mucociliary clearance, and in this way, promote proliferation of potential pathogens in the nasopharynx. It is important to emphasize that the three major pathogens that cause otitis media are very often present in the nasopharynx of the normal, healthy child, but in small numbers. These organisms consist of *Streptococcus pneumoniae*, NTHi, and *Moraxella catarrhalis*. A schematic diagram of the normal homeostatic mechanisms that probably exist in the nasopharynx in a healthy child is shown in Figure 1.

The major results of viral infection or allergic rhinitis are the increased production of mucus in the nose and nasopharynx and in the eustachian tube, increase in the number of goblet cells in the nasal and nasopharyngeal mucosa, increase in the flow of blood in the nose, and an increase of blood flow in the middle ear. The increased vascularity and mucus production leads to eustachian-tube obstruction, which is the major element in the pathogenesis of both acute otitis media and otitis media with effusion (OME). OME represents a middle ear inflammation in which bacteria play an insignificant role and in which the patient is relatively asymptomatic. The viral infection not only causes an outright loss of cilia, but a loss of the metachronal wave of the persisting cilia. Loss of mucociliary clearance results in the replication of potentially pathogenic bacteria in the nasopharynx and the migration of these bacteria into the eustachian tube against the normal mucociliary flow. Furthermore, the eustachian tube blockage leads to a gradual underpressure of gas in the middle ear space. A negative pressure results, causing transudation of fluid into the middle ear space from the vascular tissue of the middle-ear mucosa. Those children who have a normal secretory immune response have a lower incidence of otitis media because the specific secretory immune system prevents nasopharyngeal colonization of potential pathogens. In contrast, children who lack specific secretory antibody, most likely on a genetic basis, will develop recurrent otitis media more often.

Our laboratory has recently demonstrated that during the development of bacterial otitis media, there is a dramatic change in the relationship of the nasopharyngeal micro-flora between *viridans streptococci*, the normal commensal organisms, and the potential pathogens that will eventually invade the middle ear [5]. It is also possible that at this stage of development of otitis media associated with either allergic rhinitis or viral infection, many children are treated too early in the course of the disease with antibiotics. The result of this early intervention with antibiotics during the course of a viral upper respiratory tract infection is the destruction of the normal commensal flora, which function to inhibit the colonization of potential pathogens and can lead to the emergence of resistant bacteria. Thus, there is the potential danger that early intervention with antibiotics may actually remove an important part of the micro-ecologic system of the nasopharynx.

In otitis-prone children, there is increased colonization with *S. pneumoniae*, NTHi, and *M. catarrhalis* [6]. Early colonization is associated with early episodes of otitis media. Nasopharyngeal mucosal immunity is a critical determinant for the elimination of potential middle ear pathogens from the nasopharynx, and is under genetic control. In addition, antigen-specific secretory IgA in human milk may protect against otitis media by reducing nasopharyngeal colonization rates [7]. In the experimental animal model of adenovirus and NTHi, it has been demonstrated that the exact mechanism of migration of bacteria from the nasopharyngeal tonsil or mucus is via the mucus in the eustachian tube, and that the organism itself probably does not adhere to the epithelium, either to the cilia or to the nonciliated cell, but ascends from the nasopharynx via the eustachian tube by mucus [8]. Furthermore, it is now well established (by using both DNA analysis and outer membrane protein profiles) that the organism that reaches the middle ear is identical to the organism that has been present in the nasopharynx. There is usually clonal expression of the bacteria in the nasopharynx at the time of otitis media [9]. Thus, in general, if one cultures from various locations of the nasopharynx, the organism is identical in all areas of location in the adenoid (Fig. 2).

Once the organisms reach the middle-ear mucosa, there is adherence, colonization, replication, and the release of inflammatory mediators such as endotoxin and proteoylytic enzymes. These events then lead to a host response, middle ear inflammation, and ultimately, an immune response from the host. This increased inflammatory action in the middle-ear mucosa results in vascular permeability and the migration of immunocompetent cells (lymphocytes) and antigen-presenting cells (macrophages), as well as serum factors such as complement proteins. This acute inflammatory response also results in increased mucus from goblet cells, which aggravates the negative pressure in the middle ear. The significant underventilation in the middle ear now results in a significant negative pressure, which produces even more transudation. Furthermore, the mucus production in the middle ear as a result of inflammation from bacterial or viral-bacterial interaction produces thick mucoid effusion. This vicious cycle, if not cleared by the host, aided by medical or surgical therapy, may result in the production of a chronic inflammatory state.

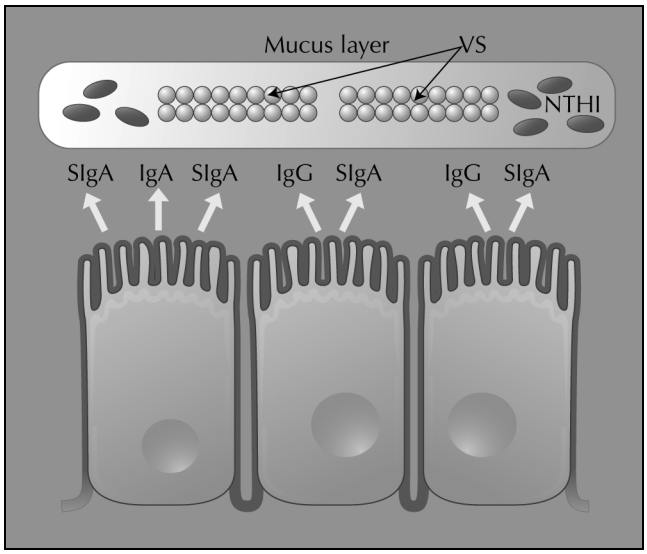

Figure 1. Bacterial micro-ecology and local mucosal immunity together maintain a healthy and "normal" bacterial flow. The nasopharyngeal mucus is demonstrated with *viridans streptococci* and NTHi in a ratio of approximately 7:1 in the normal healthy state. The adenoidal cells synthesize secretory IgA and IgG, and secrete these immunoglobulin isotypes into the mucus. These immunoglobulins suppress the colonization of potential pathogens.

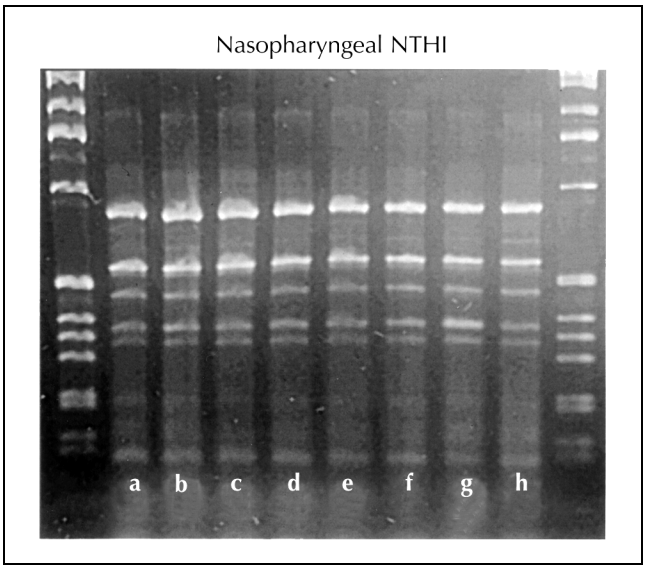

Figure 2. Nasopharyngeal colonization of NTHi. The diagram demonstrates that eight isolates taken from the adenoid of a child in eight different locations grow out an identical organism, strongly supporting the concept of clonal colonization of a strain of NTHi at any one time in the nasopharynx of a child.

Lymphocyte Trafficking in the Middle Ear in Otitis Media

Is the middle-ear mucosa part of a common mucosal immune system (mucosa-associated lymphoid tissue [MALT]), or does the inflammatory response in the middle ear result from local inflammatory chemotactic factors in the middle-ear mucosa, drawing in inflammatory cells in a nonspecific fashion? The middle-ear mucosa possesses a mucociliary system, particularly in the anterior and anterior/superior regions of the middle ear cavity near the eustachian tube opening [10].

There has been little reported information on lymphocyte trafficking into the human middle ear during otitis media. It is, therefore, speculative to consider that lymphocytes home to the human middle-ear mucosa from the gut, or tonsils and adenoids. Brandtzaeg [11••] has considered the tonsils and adenoids as upper respiratory tract Peyer's patches, and suggested that J-chain–positive IgA B-cells arise from the tonsils and adenoids and home to sites in the upper respiratory tract such as the lacrimal gland, the parotid gland, and the nasal mucosa. Our laboratory has also suggested that middle-ear mucosa may be part of MALT when inflammation of the middle ear and mucosa are present during otitis media [12]. Some of the reasoning for this is related to the fact that the tonsils and adenoids primarily possess IgA_1 as the major IgA subtype. IgA_1 is usually found in the nasal mucosa and in the upper respiratory tract, whereas IgA_2 appears to be the major IgA subclass in the gut, and therefore most of the IgA B cells that reach the lamina propria of the gut arise from Peyer's patches of the gastrointestinal tract [13••]. Although B cells destined to produce IgA can home to the upper respiratory tract from the gut in experimental animals, there is presently no evidence that homing occurs from the gut in the human with otitis media.

Experiments done in our laboratory several years ago may shed light on the role that the tonsils and adenoids play as precursors for B cells for the middle-ear mucosa [14]. We demonstrated that specific B cells are present in the middle-ear mucosa, producing IgG directed against *Streptococcus mutans*, an organism that is never found in the middle ear space in otitis media. Specific B cells with immunoglobulin receptors for various cows' milk antigens are also present in the middle-ear mucosa. These B cells are found in the tonsils and adenoids. Further support for the concept of a common mucosal immune system playing a role in the development of inflammation in the middle ear has been reported by Kurono *et al.* [15] in Japan. Enhancement of mucosal IgA responses in the middle ear cavity against NTHi follows oral immunization with these bacteria [15].

Our laboratory has also demonstrated specific IgA directed against respiratory viruses present in high concentrations in the middle-ear fluid, even when these viruses are not present in the patient at the time of fluid removal [16]. Recent investigations from our laboratory also demonstrate that the engraftment of human tonsil lymphocytes in the severe combined immune deficiency (SCID) mouse results in engraftment in the lung, but not the gastrointestinal tract [17], indicating preferential homing of tonsil lymphocytes to the upper respiratory tract rather than to the gastrointestinal tract. The above information taken together suggests that during inflammation, the middle-ear mucosa appears to receive

Table 1. Lymphocyte traffic to middle-ear mucosa

Investigators	Year	Findings
Ryan *et al.* [18]	1991	Lymphocytes seed middle-ear mucosa from vasculature in nonspecific fashion.
Kurono *et al.* [15]	1994	Both specific and nonspecific homing of lymphocytes from GALT and peripheral lymphoid tissue.
Bernstein *et al.* [14]	1988	Specific B cells in middle-ear mucosa originate from tonsils and adenoids.
Nadal *et al.* [17]	1991	Tonsillar lymphocytes engraft respiratory mucosa (lung) but not intestinal mucosa in SCID mice.

SCID—severe combined immune deficiency; GALT—gut-associated lymphoid tissue.

at least some lymphocytes from the common mucosal immune system.

In contrast to the above-mentioned investigations, Ryan *et al*. [18] concluded that homing of lymphocytes into the middle-ear mucosa during inflammation probably represents a nonspecific mechanism related to chemotactic factors that are not in turn related to specific homing. Given the rapidity with which lymphocytes appear in the middle ear cavity in otitis media, the most likely source of lymphocytes would appear to be the local vasculature. The trafficking of lymphocytes to the middle ear from various sources, both systemic and mucosal, may be nonspecific. In a model of chronic middle ear immune responses, Ryan *et al*. [18] observed that lymphocytes from the spleen, cervical nodes, and Peyer's patches entered the chronically challenged middle ear preferentially.

Therefore at the present time, it appears that the middle-ear mucosa possesses characteristics of the common mucosal immune system as well as nonspecific mucosal immunity (Table 1).

The Development of a Specific Immune Response in the Middle Ear after Bacterial Infection

The acute inflammatory response brings all of the cells into the middle ear that are necessary for a specific immune response. Although it has been demonstrated that all classes of immunoglobulins are locally synthesized in the middle-ear mucosa [19], immunoglobulins and specific antibodies enter the middle ear from the increased hyperemia of the venous blood permeating the middle mucosa. Furthermore, bactericidal antibodies of the IgG and IgM class are present and, with complement, are specific for the eradication of NTHi [20]. The most likely targets for these bactericidal antibodies are specific epitopes of the outer membrane proteins of NTHi, most specifically, P2 and P6. IgG2 subclass antibodies appear to be specific for the capsule of *S. pneumoniae*, whereas IgG_1 and IgG_3 are specific for the outer membrane proteins of NTHi [21]. If this immune response is successful in eradicating the bacteria and the other nonspecific mechanisms available to the host are capable of clearing the mucus, a normal middle ear will result without surgical intervention. However, if the middle ear remains blocked, the immune response is excessive, the organism is not eradicated, and the host response continues to result in increased mucus production, surgical intervention may be necessary in 60 to 90 days. The work from our laboratory and others suggests that IgG_1 and IgG_3 are required to interact with specific epitopes of the outer membrane proteins of NTHi. IgG_2 and IgG_4 responses are minimal [21]. Furthermore, IgG response to the lipo-oligosaccharide of NTHi does not appear to be important in the eradication of this bacterium [21,22]. Appropriate antibody synthesis requires adequate genetic background for a response. If a child has defective genes that are responsible for the synthesis of immunoglobulins, it is likely that he or she will be prone to otitis.

Although secretory IgA is critical for mucosal host immune response in the nasopharynx, its role in the closed middle ear space is still not known. It may very well be that high concentrations of middle-ear IgA may be protective to the bacteria and prevent bactericidal antibody from reaching the surface of the bacteria. Our laboratory has demonstrated that the highest middle ear concentrations of IgA are often found in the most longstanding chronic infections of the middle ear space [23]. The longitudinal course of the immune response against NTHi using bactericidal assays as well as determining the antibody response against lipo-oligosaccarides and specific outer membrane proteins, including P2, P5, and P6, has been studied (Fig. 3) [22]. The immune response to NTHi in otitis media with effusion is often strain-specific. The occurrence of second episodes of otitis media with effusion due to different strains of NTHi in the face of pre-existing heterologous bactericidal antibody suggests a lack of cross protection. Although local middle ear antibody declines over a relatively short period of time, serum antibody titers remain stable and may persist for years.

In summary, IgG is the major immunoglobulin in the middle ear responsible for eradication of the infection. It is always the predominant isotype in the middle-ear fluid as well as in the middle-ear mucosa. It has been demonstrated that P6 of NTHi, a 14.4 kilodalaton protein, is highly conserved among NTHi strains, and serves as a target for bactericidal antibody. Therefore, serum antibody response to P6 is crucial for the eradication and prevention of the disease. The failure of a patient to produce high levels of P6 may account for the otitis-prone condition.

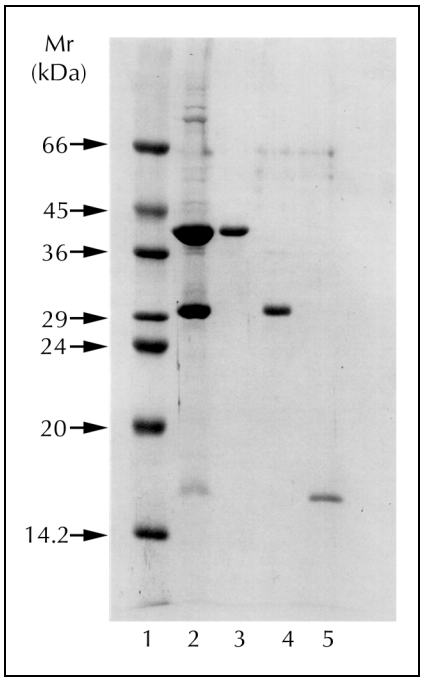

Figure 3. Isolation of three major outer membrane proteins (P2, P5, and P6) from a strain of NTHi. The outer membranes were isolated by preparative sodium dodecylsulfate-polyacrylamide gel electrophoresis (PAGE). Lane 1: molecular markers. Lane 2: outer membrane proteins of NTHi. Lane 3: purified P2. Lane 4: purified P5. Lane 5: purified P6.

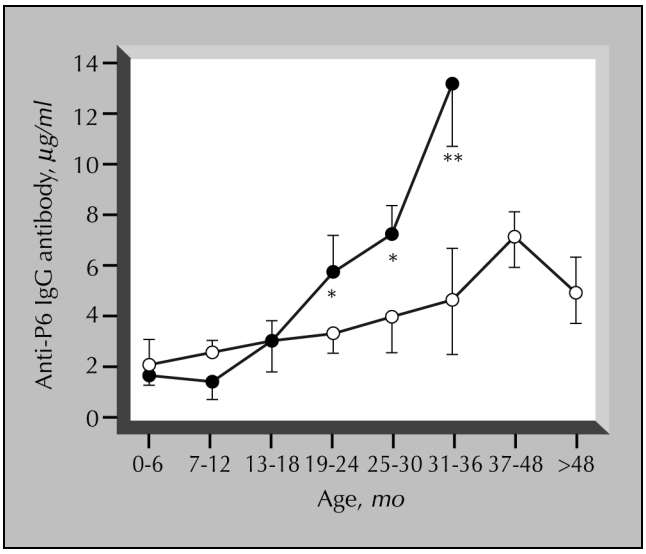

Figure 4. Longitudinal measurement of serum anti-P6 IgG antibody (microgram/millileter) of otitis-prone and non-otitis–prone children. The non-otitis–prone children have a significantly higher level of specific serum antibody directed against P6 outer membrane protein of nontypable *Haemophilus influenzae* at 19 to 24 months of age, 25 to 30 months of age, and 31 to 36 months of age.

(Fig. 4). In contrast, children who are not otitis-prone appear to make a good response to P6 over time. Taken together, these data suggest that there may be genetic reasons for the development of P6 antibodies in both serum and nasopharyngeal secretions. The former (serum antibody) accounts for bactericidal killing and resolves the infection. The latter (nasopharyngeal secretory antibody) may prevent colonization of NTHi and *S. pneumoniae* in the nasopharynx. Thus, the otitis-prone condition may reflect defects in both systemic and local immunity. It is likely that specific IgG antibody to *S. pneumoniae* capsular and membrane protein as well as specific IgG antibody to *M. catarrhalis* is most likely responsible for the resolution of otitis media with these other bacteria. The role of complement and neutrophils has not been thoroughly investigated, although it is likely that immune lysis without neutrophils is probably adequate for the resolution of otitis media NTHi and *M. catarrhalis*. It is also likely that neutrophils may be necessary for the eradication of *S. pneumoniae* associated with specific antibody and complement, and ultimate phagocytosis.

It should be noted that some investigators have suggested the immune response itself may be related to the pathogenesis of otitis media. These data are very sketchy and certainly have not been proven to date, but it should be mentioned that immune complex disease, delayed hypersensitivity, and allergic reactivity in the middle-ear mucosa have been considered responsible for the inflammatory response in the middle ear. The data from our laboratory would suggest that for the most part, at least, the immune response is protective and is responsible for the eradication of the bacteria or viruses that enter the middle ear space.

We have studied the natural history and immunology of otitis media in 526 children. Approximately 65% of these developed otitis media in the first year of life, and 13.1% were classified as otitis-prone (more than four episodes in the first year of life). As expected, *S. pneumoniae*, NTHi, and *M. catarrhalis* caused the majority of episodes of otitis media. One hundred percent of young children who were evaluated during otitis media developed an immune response to NTHi, 50% to 80% to *M. catarrhalis*, and less than 15% to *S. pneumoniae* (Type 19 examined exclusively). Children who experienced recurrent otitis media due to NTHi appeared to develop strain-specific immunity, but failed to recognize P6 as an important immunogen. Nasopharyngeal colonization with *S. pneumoniae*, NTHi, and *M. catarrhalis* was increased in otitis-prone children compared with normal children. Early colonization was associated with early first episodes of otitis media. Mucosal immunity in the nasopharynx and in the middle ear may be a critical determinate for the elimination of potential middle ear pathogens.

Conclusions

New strategies in the future for the prevention of otitis media

The bacterial organism in the middle-ear mucosa in otitis media originates from the adenoid or nasopharyngeal secretions. The resolution of otitis media by immunologic mechanisms within the middle ear space is the result of IgG subclass antibodies, particularly IgG_1 and IgG_3, and complement that reaches the middle ear space from the blood. IgA plays little or no role in the elimination of this organism once it infects the middle ear, although it is responsible for coating the bacteria and may prevent its

attachment to the middle-ear mucosa. It may actually block complement-fixing antibody, and in these ways may be antiphlogistic. Efforts to reduce the otitis-prone condition will include mucosal immune mechanisms that will prevent the colonization, or adherence, of these organisms to the nasopharyngeal mucosa.

Oral immunization may stimulate gut precursors of B cells in Peyer's patches that may seed the upper respiratory tract and produce specific IgA in the nasopharyngeal secretion. This has been demonstrated for both NTHi and *S. pneumoniae* in the experimental animal model [24]. Furthermore, there is evidence that oral immunization with bacterial vaccines may activate macrophage by stimulating metabolic and functional properties that are characteristic of the activated state and are important for host defense [25]. Systemic vaccination with specific outer membrane proteins of NTHi such as P6 or the specific fimbrial protein that may be conserved in NTHi (P5) [26] may be used to stimulate specific antibodies that may be bactericidal. However, at the present time, these vaccines are not available clinically.

Strategies to prevent bacterial infection by inhibiting adhesion of the organisms to mucosal surfaces can also be effected by the use of simple sugars. These may act as receptor analogs and may be directed against the functional group of the adhesion of the bacteria [27]. This concept has already been demonstrated in the treatment of urinary tract infections by the oral administration of cranberry juice. The concept of bacterial interference has been utilized as a method of preventing recurrent B-hemolytic streptococcal pharyngitis and tonsillitis, and there is recent support for its use in the otitis-prone condition [28,29]. Inasmuch as there appears to be an inverse relationship between *viridans streptococci*, particularly those that are inhibitory, and NTHi and *M. catarrhalis*, it is theoretically possible to consider a nasal spray or nasal suspension of *viridans streptococci* that are known to be inhibitory to prevent the otitis-prone condition.

Finally, an outstanding overview of the prevention of respiratory viral infections and immunizations against pneumococcal otitis media and nonpneumococcal bacterial etiologies of otitis media has recently been published by Klein, *et al.* [30••], and is an up-to-date review of potential vaccinations, passive immunization and other strategies for immunization against upper respiratory tract mucosal infections.

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This review includes topics involving pathogenesis of otitis media, prevention of respiratory viral infections, immunization against bacterial etiologies of otitis media and non-vaccine prophylaxis of otitis media. The authors have edited and selected materials of interest from the presentations and discussions of a conference held in France in December 2000.