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Cellular Immune Response in Young Children Accounts for Recurrent Acute Otitis Media

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Abstract

Acute otitis media (AOM) is a common disease in young children. *Streptococcus pneumoniae*(*Spn*) and *Haemophilus influenzae* (NT*Hi*) are the two most common pathogens that cause AOM. Over the past 5 years our group has been studying the immunologic profile of children that experience repeated AOM infections despite tympanocentesis drainage of middle ear fluid and individualized antibiotic treatment; we call these children stringently-defined otitis-prone (sOP). Although protection against AOM is primarily mediated by ototpathogen-specific antibody, our recent studies suggest that suboptimal memory B-& T- cell responses and an immaturity in antigen presenting cells may play a significant role in the propensity to recurrent AOM infections. This review focuses on the studies performed to define immunologic dysfunction in sOP children.

Keywords

Acute otitis media; Cellular immune response; Recurrent otitis media; Streptococcus pneumoniae; Haemophilus influenzae; Memory T cells; Memory B cells; Cytokine response; Dendritic cells

Introduction

Acute otitis media (AOM) is the most common infectious disease among infants and young children that causes temporary complications in hearing ability within the USA and this infection can take a heavy toll in developing countries as evident by 50,000 deaths/year in younger children suffering from the exacerbated form of this infection[1-3].About 60-70% of children experience at least one episode of AOM during the first three years of their life. A subpopulation of children representing30% of the total, have been found to suffer from three or more episodes of AOM within six months or four infections within a year and are considered traditionally-defined otitis-prone[4].

In 2006 our group commenced on a multi-year prospective, longitudinal study supported by NIH NIDCD to identify immunologic factors contributing to the otitis-prone condition. We have reported on microbiologic aspects of the project[5-18].Our results are unique compared to prior studies because for every episode of suspected AOM a tympanocentesis was performed to confirm the diagnosis bacteriologically. This absolutely assured that our studies only involved bona fide cases of bacterial infection in the middle ear. We attribute our success in identifying numerous new immunologic features of AOM infections to the

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strict definition of AOM cases. Moreover, after draining the middle ear of pus and inflammatory fluid (itself therapeutic) we identified the organisms involved in every case and tested the bacterial strains against a panel of antibiotics to assure that the optimum antibiotic was given to the child in every case. We term this management "individualized care" [19]. The children who experience 3 episodes of AOM within a 6-month time frame or 4 episodes within a 12-month time frame despite individualized care we term stringently-defined otitis prone (sOP)[19]. By confining our immunologic studies to this sOP cohort and making comparisons to non-sOP children we have made several new observations[20-30].

Streptococcus pneumoniae (Spn) and non-typeable *Haemophilus influenzae* (NT*Hi*) are the two most common pathogens causing AOM and work from our laboratory as well as others have demonstrated that developing antibody-mediated immunity to these pathogens is a cardinal step in preventing recurrent AOM infections in young children[14, 26, 27, 31, 32]. However, neither antibody-mediated nor cellular immunity in young children is at par with adults, which predisposes these young children to enhanced susceptibility to recurrent infections.

In this review, the divergence of cellular immune response to AOM otopathogens in infants and young children, which we have found contributes to the susceptibility of the pediatric population to recurrent AOM, is discussed. In addition due to the longitudinal aspect of our study design we have gained new knowledge regarding the progress of "developing" immunity in the age range of 6-36 months. We will also discuss evidence regarding a relatively delayed maturation of immunity in sOP children that contributes to generalized suboptimal immune responses[33, 34].

T cells mediate immunity to common pathogens of acute otitis media

During the onset of an infection, memory $CD4^+$T cells can be generated from naïve/effector $CD4^+$T cells, with memory lymphocytes populating lymphoid and non-lymphoid sites[35-37]. $CD4^+$ T-cells comprise functionally distinct populations characterized by specific cytokine profiles produced in response to antigens[38]. Primarily, $CD4^+$ T cell subsets, based on their ability to produce IFN-γ and IL-4 are defined as T-helper1 (Th1) and Th2[39]. More Th-subsets have been identified in recent years such as Th17, Th9 and Tfh based on their unique function and distinct transcription regulation [40-42].

Various reports, including ours, demonstrate a significant role of$CD4^+$Th cell subsets in providing immunity to *Spn* andNT*Hi*, the two most common otopathogens of AOM[43]. In older children (median age 5 years) and adults, antigen-specific $CD4^+$ T-cells have been shown to reduce *Spn* nasopharyngeal colonization[44, 45]. An effective pathogen-specific T-cell response in adults has been associated with protection from invasive *Spn* disease (invasive pneumococcal disease, IPD) and chronic obstructive pulmonary disease (COPD) caused by *Spn* and NT*Hi*, respectively[46, 47]. Morerecently,Th17 cells secreting IL-17, IL-21, and IL-22have been described to impart antibody-independent protection in a mouse model of pneumococcal infection[48]. Also, $CD4^+$T-cell proliferation in cells 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 OP children lack pathogen specific T cells[49].

B and T cell responses to common pathogens of acute otitis media in otitis-prone children

Recently, we found a lower percentage of *Spn* antigen-specific memory B cells among sOP children compared to non otitis-prone children[22]. The lower percentage of memory B cells in sOP children was associated with reduced levels of pneumococcal-specific IgG in their respective serum. Furthermore, using six pneumococcal and three NT*Hi* protein antigens,

we enumerated *Spn* and NT*Hi*-specific functional $CD4^+$ T-helper memory cell subsets (Th-1, Th-2 and Th-17) in the peripheral blood of a cohort of non-otitis prone and sOP children. We found a reduction in the functional memory $CD4^+$ T-cell frequencies producing various cytokines among sOP children experiencing AOM infections[21]and postulated that the reduction in antibody responses to pathogens in sOP children may be due to poor T-cell help [26, 27].Therefore, it may be that in the absence of adequate pathogen-specific memory $CD4^+$T-cellfrequencies, and after briefly-elicited antibody levels wane, the sOP child quickly becomes susceptible to additional AOM infections. In concurrence with us, earlier work in a cohort of children suffering from recurrent otitis media showed reduced frequencies of T cells producing IFN-γ in the peripheral blood as well as adenoids [50]. The authors concluded that the reduced capacity of the adenoidal T cells to produce IFN-γ might induce susceptibility to recurrent AOM infections. In another study, recurrent otitis media children were shown to have lower numbers of “active T cells” that were enhanced to comparable levels as a control group after transfer factor therapy [51].

Immunopathology of otitis media suggests role of memory T cells in controlling middle ear infection

The cellular phenotyping of MEF as well as adenoids during AOM has indicated a large migration of CD45RO+/CD45RA- memory $CD4^+$ T-cells as determined by loss of homing receptors L-selectin[52, 53]. Being a lymphoid organ, adenoids are the main site for naïve T-cell priming during upper respiratory tract bacterial infections and nasopharyngeal colonization[45, 49, 50,52]. Once an antigen loaded APC migrates to local lymphoid organs (adenoids), the differentiation of lymphocytes (*c.f.* $CD4^+$ T-cells) takes place. After entering the blood circulation the $CD4^+$ T-cells may eventually migrate to the middle ear mucosa (in the case of AOM) and/or the upper respiratory tract (during NP colonization)[54].

Several studies in rodent animal models in the past described a surge in the immunocompetent cells (*c.f.* T-, B- cells, macrophages, dendritic cells and natural killer (NK) cells) and antibodies into the MEF and middle ear mucosa after the onset of AOM [28, 53, 55, 56]. T-cells were described as dominant among the lymphocytes in the MEF during AOM, with $CD4^+CD45RO^+$ memory T-cells predominating[53]. In one rodent experimental model of AOM, it was shown that the inflamed middle ear and especially the Eustachian tube mucosa are the destination of several immune cells including T cells and the inflamed microenvironment is supportive of local proliferation of these immune cells [55]. A study in humans demonstrated that the adenoid participates in the development of memory $CD4^+$ T cell pool during allergy and otitis media [57]. Bernstein et al. reported that adenoidal cytokine profiles skew more towards Th-2 type during recurrent otitis media and they postulated that immune modulation contributed to the inflammation of the middle ear [58]. Conversely, our hypothesis is that otopathogen-specific T-cell memory, if generated, primarily is effective in immune protection by activity in the nasopharynx and the Eustachian tube, with most cells originating from regional lymph nodes, i.e., the tonsils and adenoids. In support of that notion we have shown that the antibody present in middle ear fluid predominantly, if not exclusively, derives from the serum by transudation and reflux of antibody produced in the nasopharynx that reaches the middle ear by way of the Eustachian tube[28] and that MEF of children with AOM is largely devoid of lymphocytes (unpublished).

Relative Immaturity in the dendritic cells of otitis-prone children

Dendritic cells (DC), the most potent antigen presenting cells, are the primary activators of naïve T cells. The crosstalk between DCs and naïve T cells provides the opportunity for antigen recognition through T-cell receptor (TCR) interactions with peptide-MHC complexes that are present at the DC surface [59]. TCRs of naïve $CD4^+$ T cells recognize

peptides in context to MHC II [60]. Upon antigen-uptake, DCs mature and up-regulate several accessory molecules, for example- MHC, CD80, and CD86 etc., required to efficiently prime naïve T cells [61, 62]. This fate of naïve T cell priming into effector/memory responses is also dependent on the cytokine milieu provided by matured DCs and results from toll like receptor (TLR) triggering [63]. In the same context, recent reports have shown that traditionally-defined OP children have distinct expression of pro-inflammatory cytokine (TNFa, IL-6, IL-10) expressing genes that may be consistent with a relatively immature immune system [64, 65]. We too observed differences in the genetic pattern of NTHi-caused AOM in sOP children [24]. Also a different regulation of IL-10 cytokine exists during AOM [25]. We recently discovered that a diminished innate inflammatory response exists in sOP children [30]. Since DCs link the innate immune system and the adaptive immunity by such features as PAMPs and T-cell activation (TLRs, cytokines), adequate priming of naive T-cells and generation of effective memory T-cells may be compromised in the sOP child by inefficient APC function. Therefore, we sought to determine if sOP children have an immature pool of DCs that impairs the generation of effector/memory $CD4^+$ T-cells. Our, preliminary data suggest that DCs of sOP children have significantly reduced levels of MHCII molecules on their surface (Figure 1).

Delayed age-dependent immunologic maturation in OP infants and young children

The susceptibility of infants to AOM infections wanes with age due to immunologic maturation. We followed an age-dependent comparison in the pathogen-specific IgG levels of infants and young children over time. In our studies, comparing acute to convalescent titers after AOM, sOP children had no significant change in total IgG responses to three NT*Hi* proteins (protein D, P6 and OMP26), while non-sOP children had significant increases to Protein D. Anti-protein D, P6 and OMP26 antibody levels measured longitudinally during NP colonization between the age of 6 to 24 months in sOP and non-sOP children demonstrated subtle anti-protein D IgG increases over time in sOP children compared to more than four-fold increases in the non-sOP children[26]. Furthermore in a separate study, IgG antibody titers to five proteins of *Spn* (PcpA, PhtE, PhtD, Ply and LytB)were significantly different among children over time. Characterization of IgG and IgM acute and convalescent serum antibody levels of *Spn* AOM infection showed the kinetics of the response differed among children, with the same rank order of antibody levels over time[66]. Individual data showed that some children responded to AOM with an antibody increase to one or more of these *Spn* proteins but some children failed to respond at all. We conclude that antibody levels to *Spn* proteins PcpAPhtD, PhtE, Ply and LytB, all rise over time in children age 6 to 30 month following natural exposure to *Spn* after NP colonization and AOM; however, there were significant differences in quantity of antibody elicited among these potential vaccine antigens. At their AOM visit, anti-PhtD, -LytB, -PhtE, and -Ply IgG antibody titers in sOP children were significantly lower compared with non-sOP children. Although non-sOP children had significant increases between 6 and 24 months of age in anti-PhtD, PcpA, PhtE, and Ply IgG antibody titers as a consequence of nasopharyngeal colonization and AOM, sOP children either failed to show rises or the rises were significantly less than the non-sOP children[27].

Immune responses to vaccine antigens in otitis-prone children

In previous studies, a poor immunological response among traditionally-defined OP children was proposed on the basis of reduced IgG responses. Prior studies of OP children have demonstrated mixed results when IgG responses to vaccines have been assessed. An antibody response to vaccine antigens in traditionally-defined OP children was compared to non-OP group of children and a difference in rubella specific antibody was reported [67]. Antibody levels have been measured to be low to rubella but not to DT or TT [67] or DT and TT but not PRP and measles [68]. The response to serotypes 6B, 14, 19F and 23 after

polysaccharide-conjugate vaccine were normal in a prior study of OP children[69].However no precise mechanisms were explored to confirm the observations.

We hypothesized that sOP children might have a broader immune dysfunction in eliciting optimal immune-responses to antigens. To evaluate this, we also measured IgG levels to several routine pediatric vaccine antigens: diphtheria toxoid (DT), tetanus toxoid (TT), pertussis toxoid (PT), pertussis filamentous hemagglutinin (FHA), pertussis pertactin (PRN), polio, hepatitis B (HepB),*Haemophilus influenzae type b* capsule (PRP) and pneumococcal polysaccharides. Interestingly, sOP children had undetectable or poor responses to more than half of the vaccine antigens studied after the primary series of vaccinations. We found that after vaccination, antibodies were detectable among a proportion of sOP children suggesting they may develop short-lived B-cell antibody responses post vaccination, similar to what we observed following *Spn* and NT*Hi* nasal colonization and AOM. About one-quarter of sOP children persisted with sub-protective antibody levels after first boosters at around 18 months of age [70].

Additionally, we assessed memory $CD4^+$ T-cell responses to DT, TT and PT (DTaP) vaccine antigens in age-matched cohorts of sOP and non-sOP children and found deficiencies in T-cell function and memory generation[71].However, after SEB stimulation, similar percentages of functional memory $CD4^+$ T-cells were observed in both sOP and non-sOP children. Whether these reduced IgG and T cell responses to vaccine antigens makes them susceptible to vaccine-preventable infections is difficult to establish in the U.S. because our country has high herd immunity. However, we recently found that sOP children develop neutralizing antibody to influenzae vaccines less often than non-sOP children and this failed immunologic response is followed by a very significant increase in influenzae infections (Verhoeven et al, submitted) or reduced IgG levels are otherwise enough to offer protection in this populations remains to be established. As sOP children age and receive more booster doses of vaccines a robust T-cell memory response typically develops around age 3 to 5 years[72]. Whether the sOP child “outgrows” their neonatal-like immune profile as they do in their propensity to recurrent ear infections during this age time frame is the subject of active study by our group.

Conclusions

A detailed comparison of immunologic features among young sOP and non-sOP children suggests that immune dysfunction in the sOP children resembles a more immature “neonatal-like” profile (Figure 2). This could be the underlying mechanism of reduced B-cell and T cell memory responses and diminished APC function in sOP children.

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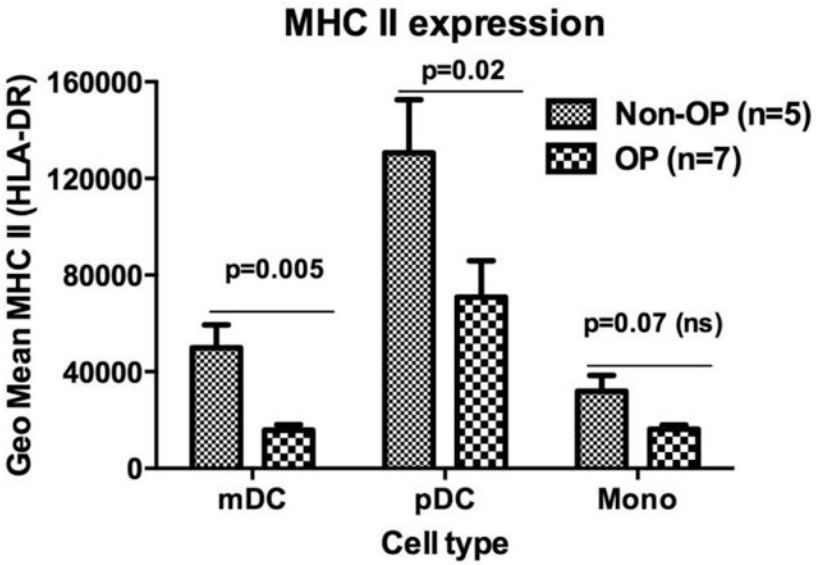

Figure 1.
MHC II expression levels in the peripheral blood of otitis-prone and non otitis-prone group of children were measured using flow cytometry. mDC (myeloid dendritic cells, pDC (plasmacytoid dendritic cells) and mono (monocytes)

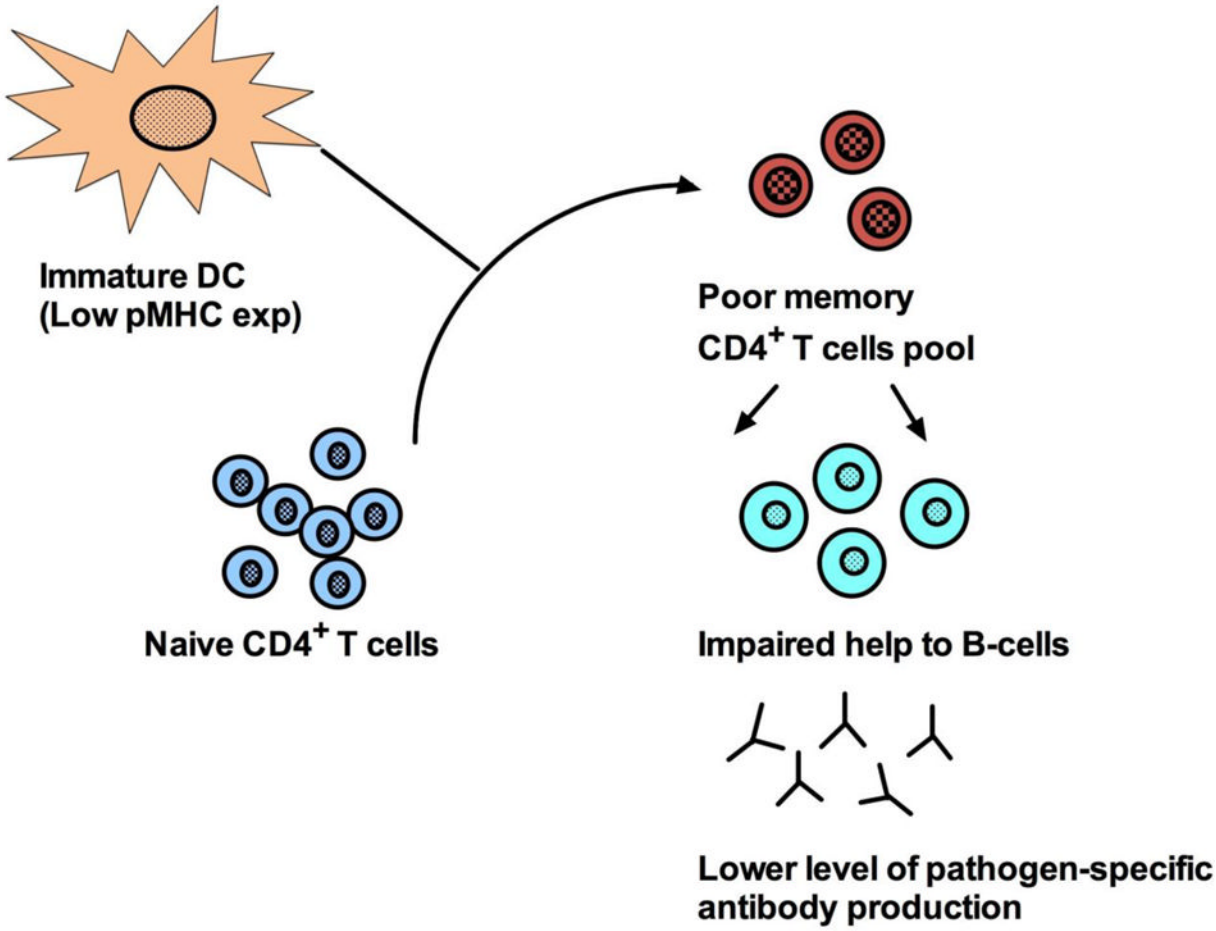

Figure 2.
Factors governing immune competence and poor antibody generation in otitis-prone (OP) group of children. DC (dendritic cells), pMHC (peptide-Major histocompatibility complex).