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ROLE OF RESPIRATORY SYNCYTIAL VIRUS IN ACUTE OTITIS MEDIA: IMPLICATIONS FOR VACCINE DEVELOPMENT

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Abstract

We summarize herein the results of various virologic studies of acute otitis media (AOM) conducted at our site over a ten -year period. Among 566 children with AOM, respiratory syncytial virus (RSV) was the most common virus identified in either middle ear fluid or nasal wash; it was found in 16% of all children and 38% of virus-positive children. Seventy-one percent of the children with RSV were one year of age or older, which was significantly older than all other viruses combined ($P = 0.045$). RSV infection was associated with the common bacterial pathogens causing AOM. Past efforts to develop vaccines for RSV have emphasized prevention of lower respiratory tract infection in infants, which is a more serious problem but less common than AOM. Our results suggest that RSV vaccines that work only against infection in older children may have value in preventing AOM, the most common pediatric disease.

Keywords

otitis media; respiratory syncytial virus; vaccine

INTRODUCTION

Acute otitis media (AOM) is the most common cause of physicians' office visits for children ages 1-4 years in the United States [1]. Some of these children develop hearing deficits resulting in poor school performance and often require surgical procedures to improve middle ear drainage. Moreover, children with recurrent AOM require frequent use of antibiotics, making AOM the most common reasons for antibiotic prescription in the United States [2]. As a result, AOM treatment has also contributed to the rapid emergence of multi-drug resistant bacteria. Direct and indirect costs of AOM are estimated to exceed 4 billion dollars per year in the United States [3].

Respiratory viruses play a major role in the pathogenesis of AOM, it is now believed that most AOM episodes occur as complications of viral upper respiratory infection (URI) [4]. Several studies have shown that RSV is the most common virus associated with AOM as well as lower respiratory tract disease in young infants [5-11]. The past efforts at development of RSV vaccines have largely targeted the short term benefit against lower airway diseases seen during primary RSV infection, such as acute bronchiolitis and viral pneumonia in infancy. In these efforts, the investigative community has largely ignored to value of prevention of recurrent

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RSV infections which manifest as less severe diseases such as URI. It is well known that URI in children can result in bacterial super-infections of the upper airway such as AOM and sinusitis. These diseases, although less severe, are far more common than RSV infection of the lower airway [12].

Experience with influenza virus vaccine in reduction of AOM has been encouraging. Therefore, reduction in RSV-associated AOM with vaccination seems plausible. We herein report a review of our virologic studies of AOM conducted over a ten-year period to provide an insight into the significance of RSV in AOM and the potential benefit of development of RSV vaccines for prevention of URI due to RSV and RSV-associated AOM. We believe this is the largest series of studies on viral etiology of AOM reported to-date from the United States.

MATERIALS AND METHODS

Patients

The study group included 566 children (aged 2 months to 7 years) with AOM who were enrolled in studies which included tympanocentesis and comprehensive virologic investigations in period between 1989 and 1998 [13-16]. All children were generally healthy children seen at our primary care clinics. None of the patients had received antibiotics during the preceding week. The diagnosis of AOM was based on acute symptoms of fever, irritability or earache, signs of inflammation (red or yellow color or bulging) of the tympanic membrane, and the presence of middle ear fluid (MEF) as documented by tympanocentesis. Children with underlying disease or co-morbidity other than URI were excluded from the studies. Informed consent was obtained from the parents or guardians of all children, and all procedures conformed to the guidelines established by the United States Department of Health and Human Services and the Institutional Review Board of the University of Texas Medical Branch.

Specimens

At enrollment (visit 1), MEF was obtained from all children for bacterial and viral studies by needle tympanocentesis as described previously [13]. A nasal wash (NW) specimen and venous blood were also obtained at enrollment from all children for viral studies and serologic studies (acute titers), respectively. Follow-up visits were scheduled 2 to 5 days after initiation of therapy (visit 2), at the end of therapy (visit 3, days 9 to 12), and approximately 1 month after initiation of therapy (visit 4). During visit 2, a second tympanocentesis was performed routinely and a second nasal wash specimen for viral studies was also collected. Venous blood was obtained at visit 3 for determination of antibody responses to viruses (convalescent titers).

Bacteriologic and virologic studies

A sterile culturette swab was dipped into the MEF samples and then streaked onto blood, chocolate, and MacConkey agar plates and dipped into meat broth for bacterial cultures. NW and MEF diluted with 1 ml of viral transport media were processed for viral cultures by inoculation into two tubes each of primary monkey kidney cells and human fibroblasts (MRC-5). In addition, Hep-2 cells or buffalo green monkey kidney cells were used during RSV or enterovirus seasons, respectively. Inoculation into cell cultures was performed within 2 to 3 hours of specimen collection. The final identification of viruses was done by standard methods.

Rapid viral antigen detection was performed on both MEF and NW specimens. For MEF, detection of RSV was performed by enzyme immunoassay (RSV EIA Diagnostic Kit, Abbott Laboratories, North Chicago, IL, or Ortho RSV Antigen ELISA Test, Ortho Diagnostic Systems, Inc., Raritan, NJ). For NW specimens, the cell pellet from the centrifuged specimen was smeared onto microscope slides and used for rapid antigen detection by indirect fluorescent

antibody techniques using commercially available antisera for RSV, influenza A and B viruses, parainfluenza types 1, 2 and 3 viruses, and adenoviruses (Bartels Viral Respiratory Panel, Baxter Healthcare Corp., West Sacramento, CA). All of the subtypes of parainfluenza, influenza and adenoviruses were combined into the respective main virus group. Viral serologic studies for the same viruses were performed by indirect fluorescent antibody technique using clinical isolates from the same season as reference antigens.

Definitions

The viral respiratory pathogen was detected by either viral culture or antigen detection tests of either MEF or NW samples obtained during visits 1 or 2 (ie. any sample at any study visit). Also, a fourfold or higher rise in viral titers between acute and convalescent sera was considered as proof of the viral infection. When calculating the rates of detection of different viruses in the MEFs, dual viral infections observed in 9 children were regarded as separate viral infections for the purpose of this study. In 25 children with positive viral findings in the MEF, the specimens from the left and right ears had been combined before viral culture or antigen detection test. These specimens were excluded from the analysis of specific viral-bacterial combinations in the middle ear.

Statistical analysis

Comparison of proportions between the groups was done by the standard chi-square test and Fisher's exact test. The Mann-Whitney U test and Kruskal-Wallis one-way analysis of variance by ranks were used to compare nonparametric continuous data between the groups. A *P*-value of less than 0.05 was considered to indicate statistical significance.

RESULTS

Of 566 children with AOM included in this study, 55% of children were males. The mean age of children was 20 months, the median age was 14 months (Table 1). The distribution of ethnicity/race represented the overall distribution of patients seen our general pediatric clinics. Demographic characteristics of children with RSV infection, other viral infections, bacterial alone and no pathogen cases are summarized in Table 1. AOM risk factors such as family history of asthma and allergies, or environmental factors such as number of children in the household, exposure to cigarette smoke, daycare attendance, pets, etc. were not analyzed because the data were not consistently collected.

Viruses associated with AOM

Using viral culture and viral antigen detection tests of NW and MEF samples, and virus-specific serologic tests, 239 children (42%) were identified to have concurrent viral infection during the AOM episodes; 28 (12%) of these children had more than one virus infection (Table 2). Of the 269 viruses identified by study of NW, MEF and serology (Table 3), NW samples accounted for the most frequent virus-positive source (183 of 269, 68%), while MEF accounted for the second most frequent source (119 of 269, 44%). Thirty-two viruses (12%) were identified by serology only; 5 of these were also co-infected with other viruses as detected by culture and/ or antigen detection tests.

RSV was the most common virus identified, it was found in 90 children, which comprised 16% of total 566 children and 38% of virus-positive children. RSV-alone cases (without any other virus) accounted for 31% of 239 virus-associated cases. Parainfluenza, influenza, enterovirus, adenovirus, and rhinoviruses were identified in descending order of frequency. RSV was the most common virus (*n* = 15) identified in association with another virus. Concurrent infections with cytomegalovirus (CMV) and herpes simplex virus (HSV) were found in 26 and 3 cases,

respectively. In MEF, RSV was the most frequent virus detected, accounting for 55% of all virus-positive MEFs; indeed, RSV was identified more frequently in MEF than in NW samples.

Viruses and bacteria in MEF

A total of 982 MEF samples from 566 enrolled children were studied. Three major bacterial pathogens were isolated from MEF samples of 698 ears (71%): *Streptococcus pneumoniae* from 253 (26%), *Haemophilus influenzae* from 229 (23%), and *Moraxella catarrhalis* from 129 ears (13%). Eighty-seven ears (9%) had either two or all three of the bacterial pathogens at the same time. Fifty-five (6%) of these ears had viruses as the sole pathogen without bacteria: 34 with RSV, 10 with parainfluenza viruses, 4 with influenza, 4 with CMV, 1 each with enterovirus, adenovirus, rhinovirus and HSV.

In 85 (9%) ears, both virus and bacteria were detected. RSV was detected more frequently with *H. influenzae* (48%), parainfluenza viruses were detected more frequently with *M. catarrhalis* (60%), and influenza viruses were detected most frequently with *S. pneumoniae* (100%, with or without other bacteria). Table 4 shows data from a selected subset of 67 MEF samples that contained only the three major viruses and bacteria. There was no significant association between any specific virus and bacteria, however, the sample size is very small for meaningful analysis.

Age distribution

There was no significant difference in age between children with concurrent viral infection (mean age = 21 months) and those without documented viral infection (mean age = 19.9 months; $P = 0.42$, data not shown). Of 90 patients with AOM associated with RSV infection (see Table 1), 26 (29%) were under 12 months, 34 (38%) were between 12 months and 23 months, 2 (2%) were 2 years old, 1 (1%) was 3 years old, 8 (9%) were 4 years old and older. Children with AOM and RSV were significantly older than children with AOM and other virus infection overall ($P = 0.045$).

Duration of illness prior to AOM development

Duration of respiratory symptoms prior to diagnosis of AOM by virus-type is shown in Table 2. Adenovirus infection was associated with the longest duration of symptoms prior to AOM diagnosis (mean = 8 days, median = 5 days). RSV infection was associated with the second longest duration of symptoms (mean = 5 days, median = 3 days); all other viruses presented with AOM within first 4 days of illness. The number of previous episodes of AOM prior to the study episode of AOM was comparable among all viruses. Gender and ethnicity/race did not differ significantly with respect to illness duration and viral pathogen types.

Seasonality of AOM

Analysis of virus-associated AOM cases by month of the year (Figure 1) showed that 86 % of cases occurred between the months of September and April, which corresponds to the fall / winter seasons in the Northern Hemisphere. While sporadic cases of RSV could be identified in the summer months, outbreak of RSV-associated AOM began in September and reached high peaks from January to March; during these 3 months, RSV accounted for almost half of all virus-associated AOM cases. In these same months, influenza accounted for 9% of virus-associated AOM cases.

DISCUSSION

This report represents the largest number of children with AOM for whom tympanocentesis and comprehensive virologic studies were performed in the United States. Our results show that RSV infection is more frequently found in children with AOM than any other virus.

Children with RSV-associated AOM were older than those with AOM associated with other viruses. Children with RSV infection were similar to those with other virus infections with respect to the duration of URI symptoms prior to AOM diagnosis and previous number of AOM episodes. The bacterial pathogens of AOM associated with RSV are similar to those seen with other

In our studies, we detected viruses in the MEF by viral culture and RSV antigen detection; we did not use antigen detection method for other respiratory viruses in the MEF due to the limited sample volume. It is possible that we under-detected other viruses in the MEF relative to RSV. However, the use of antigen detection test for RSV in both MEF and NW was essential because unlike other respiratory viruses, RSV is relatively labile leading to low sensitivity of viral culture compared to that of various antigen detection techniques [12]. In the study of NW samples, we only had one case of influenza virus and two cases of adenovirus that were detected only by the antigen detection tests but not by culture. This result suggested that application of antigen detection tests for influenza and adenoviruses for studies of MEF would not have yielded significantly higher identification rates of identification for these viruses.

Our study was not optimized to study other ‘difficult-to-grow’ viruses such as rhinoviruses and metapneumoviruses. Newer, sensitive tools such as reverse transcriptase-polymerase chain reaction (RT-PCR) can improve the diagnostic capabilities. Using multiplex RT-PCR, we have previously studied MEFs for RSV (A and B strains), influenza A and B, and parainfluenza viruses 1, 2 and 3 [17]. We detected viruses in 29 of 46 (63%) of MEF samples that had tested negative for viruses by culture and RSV antigen detection tests; six of these samples were positive for RSV by the multiplex PCR. In a study of Finnish children, Piktaranta et al specifically evaluated the role of rhinoviruses, RSV and coronaviruses by PCR in 92 children with AOM; these viruses were detected in MEFs and nasal aspirates of 20%, 13% and 5% of children, respectively [18]. In another Finnish trial reported by Nokso-Koivisto et al, PCR was performed on MEF and NW samples to detect rhinoviruses, enteroviruses and parechoviruses, and antigen test was used to detect RSV; rhinoviruses and RSV were two most common types of viruses found [11]. This result suggests that in some communities, and depending on the detection techniques employed, rhinoviruses may be more common than RSV in children presenting with AOM.

Nevertheless, the best technique to identify viruses will always be subject to certain limitations. For example, the PCR assays for various viruses have different sensitivity and specificity, and the samples may contain inhibitors of PCR reactions. We believe that the virus positive samples identified by lower sensitivity assays such as culture and antigen positive represent samples that contain higher titers of virus which in turn may have a stronger association with AOM. Nonetheless, if we had applied PCR methodologies to our samples, the possibility remains that virus(es) other than RSV may have ranked higher as a risk factor for AOM.

The risk of development of AOM after viral URI has been well studied. Ruuskanen et al have shown that 57% of children with RSV infection develop AOM as compared to 35% for influenza A virus; other viruses had lower rates of AOM.⁷ Henderson et al showed a somewhat lower risk of AOM (33%) following RSV infection, however, it was still the most common virus associated with AOM [8]. These results suggest that not all respiratory viruses may be equal in predisposition towards AOM. The pathogenetic mechanisms involved in development of AOM that differentiate RSV from other viruses are unknown. Together with our results, the published data to-date indicate that an effective vaccine against RSV, as opposed to other respiratory viruses, is likely to have the highest impact against the occurrence of AOM following URI.

Our results show that RSV in association with AOM occurs in children with a mean age of 22 months and with the upper limit of 79 months, suggesting that many of these children likely had previous infections with RSV. Glezen et al have shown that immunity to RSV infection is not long-lasting and re-infection is common; 68% of children have primary infection in the first year of life of which 75% of them are re-infected in the second year of life [19]. The rates of re-infection by age 3, 4 and 5 years are 45%, 33% and 50%, respectively [19]. Most of the re-infections, however, are restricted to the upper airway. Our previous studies and those by other investigators suggest that recurrent infection of the upper airway with RSV leads to AOM in older children [20-21]. Therefore, RSV vaccines for AOM would need to show efficacy against recurrent RSV infection but not necessarily need to be effective against primary RSV infection in young children. Indeed, the strategy to vaccinate older children may overcome the problem of ineffective immune responses to RSV vaccines in young infants which is in part due to the presence of maternal antibodies and the relative immaturity of the immune system [22].

The benefit of using viral vaccines to prevent AOM has been demonstrated clearly in several clinical trials of influenza vaccines in children. Heikinnen et al reported 83% reduction in AOM in association with influenza A infection in children immunized with inactivated trivalent influenza vaccine [23]. Using a similar vaccine, Clements et al reported 32% reduction during influenza outbreak in vaccinated children [24]. Hoberman et al showed a 62% reduction in influenza-associated AOM among vaccinated children [25]. The efficacy of live intranasal cold adapted influenza vaccine was also studied 1,602 children, in whom the vaccinated children showed 30% lower incidence of febrile AOM [26]. As influenza vaccine is now being given universally to children age 6 to 59 months in the United States, its impact on reduction of AOM in the pediatric population-at-large needs to be studied.

Conceptually, RSV vaccines for AOM prevention may have fewer problems of formulation as compared to other common respiratory viruses associated with AOM. Influenza vaccines need to contain both type A and B strains and require annual immunization because of antigenic drifts and shifts. Rhinovirus vaccines would need to cover a large number of serotypes associated with URI which have very little cross-protection among each other. The RSV vaccines may require only one formulation as the various subgroups of RSV have significant immune cross-protection and there is no antigenic shift or drift.

A proof of concept has been established regarding the possible efficacy of RSV vaccines against AOM. In a small study of 109 premature infants given passive immune prophylaxis with intravenous RSV-Immune Globulin (RSV-IG), the incidence of AOM was reduced by about 80% [27]. However, this polyclonal antibody preparation from pooled human plasma donors also contained antibodies against other viral and bacterial pathogens of AOM which did not allow the evaluation of RSV-specific protection. In contrast to RSV-IG, palivizumab, an RSV-specific monoclonal antibody preparation, was shown not to provide protection against AOM [28]; this may be due to the fact that palivizumab does not protect against RSV replication in the nasopharynx. There is optimism, however, that the next generation of monoclonal antibody preparation, motivizumab (MedImmune Inc.), which is highly effective against controlling RSV replication in the nasopharynx, may provide protection against AOM. A cold-adapted, live intranasal RSV vaccine has been tested, but this vaccine produced URI, leading to cessation of further studies [29]. Additional attenuation may therefore be needed before future studies can be conducted. Englund and Anderson have summarized the various RSV vaccines that have been tested in the past and the newer vaccine candidates that can be considered for protection against lower and upper airway disease, including AOM [30-31].

Among currently available bacterial vaccines, clinical trials of a protein-conjugate pneumococcal vaccine has shown 34% reduction of AOM due to all serotypes of pneumococci

and 56% reduction of AOM for vaccine serotype-specific AOM [32]. In the real practice situation, a recent study of a large national database has shown a significant overall reduction in AOM since the introduction of the heptavalent pneumococcal vaccine [33], a benefit not intended in the original regulatory approval of the vaccine. In view of the demonstrated benefits of pneumococcal and influenza vaccines, we strongly recommend their use for reduction of AOM in children. As there are no currently licensed vaccines for other bacterial pathogens of AOM, vaccines targeted against respiratory viruses have the advantage of providing protection against such bacterial pathogens. Furthermore, viral and bacterial vaccines together may be able to provide a better benefit than either vaccine alone. Another potential advantage of viral vaccines is that the benefit could extend beyond AOM to other bacterial super-infections that follow virus infections such as acute sinusitis, acute bacterial bronchitis, and even bacterial pneumonia.

In summary, our studies show that RSV is the most common virus associated with AOM, a disease of low severity but high prevalence. Efforts are needed to develop vaccines that protect against RSV-associated AOM and other bacterial complications. Experience with influenza virus vaccines suggests that effective RSV vaccines could better protect against AOM. There are several hurdles in developing RSV vaccines against AOM, including demonstration of effectiveness in preventing recurrent URI due to RSV in older children.

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Figure 1.

Seasonal distribution of all AOM cases, AOM associated with RSV, and AOM associated with other viruses

Table 1
Demographic data of 566 children with AOM in relation to pathogen groups

Characteristics	All Children	RSV ^a +/- Other Viruses	Other viruses ^b	Bacteria Alone	No Pathogen	P-Value
No. of children	566	90	149	213	114	
Age in mos.						
Mean (median)	20 (14)	22 (16) ^c	18 (13)	21 (14)	21 (13)	0.44 ^d
Range	2-89	3-79	2-75	2-89	2-81	
Gender (%)						
Male	312 (55)	54 (60)	79 (53)	114 (54)	65 (57)	0.68
Female	254 (45)	36 (40)	70 (47)	99 (46)	49 (43)	
Race/ ethnicity						
Asian	3 (1)	0 (0)	0 (0)	2 (1)	1 (1)	0.81
African American	211 (37)	35 (39)	52 (35)	81 (38)	43 (38)	
Caucasian	205 (36)	37 (41)	53 (36)	75 (35)	40 (35)	
Hispanic	147 (26)	18 (20)	44 (30)	55 (26)	30 (26)	

^a RSV alone in 75 cases, RSV with other concomitant viruses in 15 (see Table 2)

^b Single viral infection in 136 cases, 2 viruses in 12, and 3 viruses in 1 (see Table 2)

^c Mean age of 75 patients with only RSV infection = 23 mos. (median age 15 mos)

^d Significant difference in age (p=0.045) between 'RSV' group (n=90) and 'other viruses' group (n=149).

Table 2Types of viruses and clinical features in children with AOM with confirmed virus infection^a

Viruses	No. of Cases	Mean age in mos. (median)	Mean no. of days of URI symptoms prior to AOM diagnosis (median)	Mean no. of previous OM episodes (median)
Single viruses (n = 211)				
RSV	75	23 (15)	5 (3)	2 (2)
Parainfluenza viruses	28	17(12)	4 (3)	2 (1)
CMV	26	18 (14)	3 (2)	3 (2)
Influenza	25	31 (26)	3 (2)	4 (3)
Enterovirus	24	14 (10)	4 (2)	3 (2)
Adenovirus	23	16 (13)	8 (5)	3 (1)
Rhinovirus	7	15 (13)	4 (3)	3 (2)
Herpes simplex virus	3	11 (11)	2 (2)	2 (2)
Multiple viruses (n = 29)				
		Range	Range	Range
Adenovirus and CMV	2	11 - 12	3 - 7	1 - 2
Adenovirus and enterovirus	3	2 - 38	2 - 3	0 - 4
Adenovirus and rhinovirus	1	13	3	0
CMV and enterovirus	1	8	4	0
CMV and influenza	2	14 - 17	3 - 14	3 - 7
CMV and parainfluenza	2	10 - 36	1 - 10	3 - 4
Enterovirus and parainfluenza	1	9	7	NA
RSV and adenovirus	3	10 - 25	0 - 3	0 - 3
RSV and CMV	3	3 - 6	1 - 3	0
RSV and influenza	2	22 - 26	3 - 14	0 - 10
RSV and parainfluenza	3	18 - 25	1 - 14	0 - 14
RSV and rhinovirus	3	11 - 33	4 - 14	0 - 4
CMV, parainfluenza, rhinovirus	1	13	4	1
RSV, parainfluenza, CMV	1	14	7	4

^a26 children with 2 viruses, and 2 children with 3 viruses

Table 3

Detection of viruses by study of various samples in 239 children^a with AOM associated with concurrent viral infection

Virus	MEF at enrollment	NW sample at enrollment	Serology (≥ four-fold rise)	Virus in any sample^b
RSV	66	36	30 ^c	90
Parainfluenza	17	24	15 ^d	36
Adenovirus	3	24	13 ^e	32
Influenza	12	22	14 ^f	29
Enterovirus	5	28	ND	29
Rhinovirus	4	11	ND	12
Herpes simplex	1	2	ND	3
CMV	11	36	ND	38
Total	119^g	183	72	269

ND = not done

^a 269 viruses were detected from 239 children with AOM; virus data of MEF and NW were from samples collected at enrollment and at follow-up visits. Paired (acute and convalescent) viral serologic studies were conducted in 172 children with AOM

^b Some viruses were detected from more than one sample (enrollment MEF and NW or positive serology) from the same subject

^c 11 cases were positive by serology only, 1 of these was positive for other virus by culture.

^d 9 cases were positive by serology only, 1 of these was positive for other virus by culture

^e 6 cases were positive by serology only, 2 of these were positive for other viruses by culture

^f 6 cases were positive by serology only, 1 of these was positive for other viruses by culture

^g 119 cases had virus in the 140 MEF samples

Table 4Specific bacteria and viruses in 67 MEF samples containing both bacteria and viruses^a

Bacterial Species^b	Respiratory Syncytial Virus^c (n=51)	Parainfluenza viruses^c (n=9)	Influenza (n=7)
<i>Streptococcus pneumoniae</i>	21 (41)	3 (33)	7 (100)
<i>Haemophilus influenzae</i>	27 (53)	3 (33)	2 (29)
<i>Moraxella catarrhalis</i>	6 (12)	6 (67)	3 (43)

^a Eighty-five MEF samples contained both bacteria and viruses. Not shown in this table are 18 MEF samples containing other viruses and bacteria^b Nine MEF samples contained more than one of above three types of bacteria (8 with 2 types of bacteria, and 1 with 3)^c One MEF sample contained two viruses (RSV + parainfluenza)