Comparison of the Etiology of Viral Respiratory Illnesses in Inner-City and Suburban Infants

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(See the editorial commentary by Heymann and Platts-Mills, on pages 1331–3.)

Background. The risk of developing childhood asthma has been linked to the severity and etiology of viral respiratory illnesses in early childhood. Since inner-city infants have unique environmental exposures, we hypothesized that patterns of respiratory viral infections would also be distinct.

Methods. We compared the viral etiology of respiratory illnesses in 2 groups: a cohort of 515 infants from 4 inner-city areas and a cohort of 285 infants from mainly suburban Madison, Wisconsin. Nasal secretions were sampled during periods of respiratory illness and at 1 year of age and were analyzed for viral pathogens by multiplex polymerase chain reaction.

Results. Overall, inner-city infants had lower rates of viral detection. Considering specific viruses, sick urban infants had lower rates of detectable rhinovirus or respiratory syncytial virus infection and higher rates of adenovirus infection. Every urban site had a higher proportion of adenovirus-positive samples associated with illnesses (10%–21%), compared with Madison (6%).

Conclusions. These findings provide evidence that inner-city babies have different patterns of viral respiratory illnesses than babies who grow up in a more suburban location. These findings raise important questions about the etiology of virus-negative illnesses in urban infants and the possibility of long-term consequences of early life infections with adenovirus in this population.

Children who grow up in large, low-income urban areas are especially prone to develop asthma, and this has prompted efforts to identify urban environmental or lifestyle factors that promote this disease. Factors under investigation include exposure to allergens, such as those of cockroaches and mice; stress; diet; lack of exercise; genetics; and exposure to indoor and outdoor pollutants [1, 2]. Another factor that is strongly related to asthma is the development of virus-induced wheezing illnesses in infancy. Recently, it has been reported that wheezing illnesses caused by human rhinoviruses (HRVs) are associated with a particularly high risk of recurrent wheezing and asthma [3, 4], as is airway colonization in the first few months with specific bacterial pathogens (eg, Haemophilus influenzae and Streptococcus pneumoniae) [5]. These findings suggest that certain pathogens in early life either contribute to the pathogenesis of asthma or serve as indicators of a predisposition to this disease.

Children raised in inner-city environments may have unique patterns of infectious diseases, including a greater likelihood of infection with herpesviruses, such as Epstein-Barr virus and cytomegalovirus, at an earlier age [6, 7]. This suggests that urban babies might also have unique patterns of infection with common respiratory viruses. If this is true, this could have implications for the subsequent risk for
developing recurrent wheezing and asthma. To test this hypothesis, we used similar molecular diagnostic methods to analyze specimens of nasal mucus for common respiratory viruses from infants aged ≤12 months of age participating in 2 prospective birth cohort studies: a mainly suburban cohort of children living in or around Madison, Wisconsin [8], and an urban cohort of children from 4 large US cities [9].

MATERIALS AND METHODS

Study Populations

The Urban Environment and Childhood Asthma (URECA) Study

The ongoing URECA study is designed to test the effects of urban environmental and lifestyle factors on immune development and the risk of asthma [9]. Entry criteria included residence in one of 4 urban areas (Boston, Baltimore, New York, and St. Louis) in which at least 20% of the population had incomes below the poverty line, as well as self-reported allergies or asthma in at least 1 parent. Expectant families were recruited during the prenatal period. Exclusion criteria included perinatal respiratory distress, human immunodeficiency virus infection, or gestational age of <34 weeks. The protocol was approved by the human subjects committees of all participating centers, and informed consent was obtained before enrollment.

The Childhood Origins of Asthma (COAST) Study

The COAST study is a longitudinal birth cohort study that was designed to test for effects of immune development and early life viral respiratory infections on the risk of allergic diseases and asthma [8]. Expectant families were recruited during the prenatal period. As in the URECA study, entry criteria required that at least 1 parent have self-reported allergies or asthma. All babies were delivered in Madison-area hospitals. Babies were excluded from the study if they were born at ≤36 weeks gestation or developed significant respiratory distress or other health conditions affecting either the lung or the immune system. The COAST study was approved by the University of Wisconsin–Madison Human Subjects Committee, and informed consent was obtained before enrollment.

Scorecard to Assess Symptom Severity

A scorecard was used by COAST study coordinators to identify children who had symptoms indicative of moderate or severe colds or any lower respiratory illnesses [10]. Points were scored as follows: 1 point each was assigned for fever, mild cough, mild rhinitis, and illness duration of >4 days; 2 points were assigned for moderate cough or moderate-to-severe rhinitis; 3 points were assigned for apnea; and 5 points were assigned for wheezing, retractions, tachypnea, or cyanosis. Parents were counseled to call the study center when respiratory symptoms were noted, and coordinators administered the scorecard over the telephone. The scores were summed, and for scores of ≥5, which were deemed to signify a moderate or severe illness, study coordinators arranged for collection of nasal mucus at the home, medical office, or other location. For illnesses that lasted >2 weeks, a second sample of nasal mucus was collected.

The URECA study used a modified version of the COAST scorecard that included recommendations for utilization of health care services that were tailored to the severity of respiratory and general health symptoms (Supplementary Materials). As in the COAST study, parents were advised to contact their URECA site by telephone if symptoms of respiratory illnesses were noted, and for scores of ≥5, arrangements were made to collect a sample of nasal mucus in the home or clinic. A sample of nasal mucus was also obtained from children in both the URECA and COAST studies at the 12-month clinic visit, and respiratory symptoms were recorded using the same scorecard. Specimens were collected during November 1998–May 2001 (a 2.5-year period) for the COAST study and during February 2005–March 2008 (a 3-year period) for the URECA study.

Respiratory Illness Questionnaires

For the URECA study, additional information on respiratory illnesses was collected every 3 months by a questionnaire that was administered at the 3-month home visit, by telephone calls at 6 and 9 months, and at the 1-year clinic visit. Information was collected about the frequency and severity of colds and other respiratory illnesses.

Assessment of Self-Reporting of Respiratory Illnesses

To estimate adherence with URECA study procedures related to self-reporting of illnesses, a substudy was conducted in November 2007–January 2008, when the children were 1–2 years of age. In this substudy, 80 URECA families (20 at each site) were selected for calls every 2 weeks, during which questions were asked about respiratory illnesses. The estimated rates of illness and infection obtained in this frequent monitoring group were compared to those obtained for the entire cohort receiving telephone-based reminders every 6 weeks.

Specimen Collection

In both studies, study personnel collected nasal mucus samples by nasal lavage, using a modified bulb syringe [11]. Approximately 2 mL of saline was squirted into the nose, and lavage fluid was obtained with gentle suction. The samples were kept cold and brought to the site laboratory, where they were divided into aliquots and frozen, pending analysis for respiratory viruses.

Viral Diagnostic Testing

The COAST nasal mucus samples were initially analyzed using viral culture for common respiratory viruses, immunofluorescent antibody staining of cells for respiratory syncytial virus...
rates of viral detection in the frequently called group were similar to those in the rest of the cohort (Table 2).

Estimates of the frequency of respiratory illness in the main URECA cohort were also specified by the caretakers on quarterly questionnaires. The estimate of illness frequency in the frequently called group (1.42 illnesses per child) was comparable to those based on parental recall and recorded on quarterly questionnaires for the rest of the URECA cohort during the same period (1.18 illnesses per child during November 2007–January 2008 and 1.41 illnesses per child during January–March 2008).

### Table 2. Respiratory Illness Rates According to Frequency of Telephone-Based Ascertainment, in the Urban Environment and Childhood Asthma Cohort During a 12-Week Period Between November 2007 and January 2008

<table>
<thead>
<tr>
<th>Variable</th>
<th>Calls Every 6 Weeks</th>
<th>Calls Every 2 Weeks</th>
<th>Rate Ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colds per child, no.</td>
<td>0.35</td>
<td>1.42</td>
<td>4.05 (3.18–5.14)</td>
<td></td>
</tr>
<tr>
<td>Nasal washes obtained, no.</td>
<td>0.19</td>
<td>0.58</td>
<td>3.00 (2.10–4.27)</td>
<td></td>
</tr>
<tr>
<td>Virus-positive nasal washes, %</td>
<td>57.7</td>
<td>50.0</td>
<td>...</td>
<td>.39</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

* Standard study procedures (n = 467).

b Subset of mothers (n = 80) in the frequently called group.
Exposures Associated With Rates of Respiratory Illnesses

Several exposures were positively related to the number of colds reported at the URECA quarterly visits (Figure 1). As expected, the frequency of colds was positively related to the number of other children in the home ($r = 0.20, P < .001$) and negatively associated with breast-feeding at the age of 3 months ($r = -0.10, P = .027$). In addition, respiratory illnesses were positively associated with mouse protein in house dust from the room where the child slept ($r = 0.11, P = .016$; Figure 1), and there was a similar trend for a positive correlation with mouse protein in dust from the living room ($r = 0.09, P = .079$; data not shown). In addition, the number of respiratory illnesses was positively related to infantile eczema ($r = 0.10, P = .030$) and inversely related to the presence of any allergen-specific immunoglobulin E (IgE) at 1 year of age ($r = -0.11, P = .035$). The number of respiratory illnesses was not associated with exposure to tobacco smoke or NO$_2$ in the home; to cat, dog, dust mite (der p and der f), or cockroach allergens in dust (Figure 1); or to parental characteristics, such as maternal ethnicity, allergy, or asthma (data not shown).

Viral Detection

Overall, viruses were detected in 67.5% of samples (199 of 295) from URECA children with symptom scores of $\geq 5$ (Table 3), and the rate of virus detection increased with the severity of illness ($P < .001$; Figure 2). HRVs alone were detected most often (in 24.1% samples [71 of 295]), followed by multiple respiratory viruses (in 18.0% [53 of 295]). The most common combinations were HRV plus either adenovirus, RSV, or parainfluenza virus. HRV detection rates were

![Figure 1. Predictors for the frequency of respiratory illnesses in 515 Urban Environment and Childhood Asthma study participants. Abbreviation: IgE, immunoglobulin E.](https://academic.oup.com/jid/article-abstract/206/9/1342/825010)
highest among children with mild illnesses ($P = .027$; Figure 2B), while detection of either RSV alone ($P = .003$; Figure 2C) or multiple viral pathogens ($P < .001$; Figure 2D) were greatest among infants with more severe illnesses. In asymptomatic children (respiratory score, 0), the virus detection rate was 25.1% (56 of 223); HRV was detected most often (in 9.4% [21 of 223]), and infections with multiple viruses were unusual (4.5% [10 of 223]; Figure 2).

Compared with specimens from URECA participants, viruses were detected more frequently in specimens from COAST participants collected during moderate-to-severe illnesses (score, ≥5) (89.2% of cases [523 of 586] vs 67.5% [199 of 295]; $P < .001$; Table 3). Similar relationships were observed for HRV ($P < .001$), RSV (nonsignificant trend [$P = .07$]), and infections with multiple viruses ($P = .02$). The rate of virus detection in healthy children was also higher in the COAST study (42.4% of cases [70 of 165] vs 25.1% [56 of 223]; $P < .001$). These results were essentially unchanged when we restricted the analysis to URECA children born at ≥36 weeks of gestation, an inclusion criterion for COAST eligibility (data not shown).

In contrast to HRV and RSV infections, adenovirus infections were found more frequently in the URECA study, compared with the COAST study (Figure 3); this was true for adenoviruses detected as the sole pathogen (4.3% of cases [28 of 656] vs 1.1% [9 of 823]; $P < .001$) and for adenoviruses detected either alone or together with another virus in the same specimen (10.7% [70 of 656] vs 5.8% [48 of 823]; $P < .001$). These differences were apparent across the entire range of illness severity (Figure 3).

The percentages of adenovirus infections among infants with moderate-to-severe illness were consistently high in children from all 4 URECA sites (10%–21% of samples per site), compared with children from Madison (6% [37 of 586]), and rates were highest among New York participants (21% [5 of 24]; Figure 4). The majority of adenoviruses in both studies (94% in the URECA study and 100% in the COAST study) belonged to group C.

**Host Factors Associated With Adenovirus Infections**

We next analyzed associations between specific host factors and adenovirus infections within the URECA cohort. There were no significant associations between adenovirus infections and demographic characteristics, breast-feeding, number of other children in the home, day care attendance, income, presence of eczema, allergen-specific IgE levels, or environmental exposures to allergens, endotoxin, or tobacco smoke in the home (data not shown).

**DISCUSSION**

Children who grow up in economically disadvantaged urban neighborhoods have high rates of wheezing illnesses and...
asthma, and the reasons for this are multifactorial and could be related to maternal smoking, air and environmental pollutants, and unique patterns of allergen exposure. In addition to these factors, there is evidence that patterns of infectious diseases are also different in urban infants, and this prompted us to comprehensively compare the etiology of viral respiratory illnesses and infections in urban infants to those in suburban infants. Using similar methods for specimen collection and analysis, we found that illnesses in urban infants were less likely to be associated with common respiratory viruses, such as HRV and RSV. In contrast, infections with adenovirus, likely to be associated with common respiratory viruses, such as HRV and RSV. In contrast, infections with adenovirus, likely to be associated with common respiratory viruses, such as HRV and RSV.

The rates of overall viral recovery were positively related to the severity of illness, but for each illness category, including asymptomatic infections, viral detection rates were lower in the urban infants. There are several possible reasons for this difference. Since specimen collection methods were similar and the PCR-based viral diagnostic tests were performed in the same laboratory, technical factors are unlikely to explain the differences. It is possible that more of the urban than suburban children were infected with undetected viruses. We did not test for 3 known respiratory pathogens (the HKU1 coronavirus and 2 polyomaviruses [WU and KI]); however, these viruses account for <5% of respiratory illnesses in most epidemiologic surveys.

Alternatively, urban infants could be more likely than suburban infants to have respiratory illnesses that are caused by bacterial pathogens, such as S. pneumoniae, H. influenzae, and Moraxella catarrhalis. Previous studies have suggested a possible role for these pathogens in causing colds [13] and wheezing illnesses [14], and this concept has been reinforced by a recent prospective birth cohort study conducted in Denmark [15]. In the Danish study, detection of one of these respiratory pathogens in the first 3 years of life was associated with wheezing, and the strength of this association was similar in magnitude to that observed with detection of a respiratory virus (odds ratio, 2.9 vs 2.8). Interpretation of these findings is complicated by substantial rates of nasopharyngeal colonization (up to 25%–30%) among children.

Finally, perhaps some of the illnesses in urban infants were noninfectious and instead were caused by exposure to noxious stimuli, such as pollutants. Rates of allergen-specific IgE were low in these young children (data not shown), so it is unlikely that allergic rhinitis was a substantial contributor to respiratory symptoms.

Adenovirus was the only virus detected significantly more often in samples from urban children than in samples from suburban children. Adenoviruses most frequently cause either respiratory symptoms, gastroenteritis, or keratoconjunctivitis, and the viral diagnostic tests in our study focused on detection of B, C, and E species, which are most closely associated with respiratory illness. The severity of illness is also strain dependent; B viruses more often cause lower respiratory tract illnesses, while C viruses that were predominant in this study are more likely to cause cold symptoms. In hospital-based studies of bronchiolitis and lower respiratory tract illness in infants and young children, adenoviruses caused 7%–15% of illnesses, and in 60%–80% of these illnesses adenovirus was detected with another virus [14,16–18]. In outpatient illnesses, the contribution of adenovirus infection is more variable. Kusel and colleagues reported virology data for the first year of life in 263 children participating in a birth cohort study in Perth, Australia [19]. Adenoviruses were detected infrequently in either symptomatic infants (1.4%) or asymptomatic infants (0.4%), and these findings were similar to those from a second study of infants studied as outpatients in the United Kingdom [20]. Notably, the demographic characteristics of the Perth cohort closely resemble those of the COAST cohort, in which white race and mid-to-high socioeconomic status predominated. In contrast, one large study found higher adenovirus detection rates among young children studied as outpatients.

Chomnaimtree and colleagues studied 294 children aged 6 months to 3 years in Galveston, Texas, and found adenovirus by PCR in 24.1% of samples obtained during colds (including 13% of samples in which adenovirus was the only pathogen) [21]. The reason for the high rate of adenovirus infections was not stated, although it is of interest that the demographic characteristics of the study population (56% Hispanic or Latino and 31% Black) were more similar to the URECA cohort than to the COAST cohort. A number of children had adenovirus detected more than once, and sequence analysis showed that, in 81% of cases, this represented a chronic...
infection rather than reinfection with a different adenovirus strain [22].

Subtype B1 adenovirus strains (eg, adenovirus 14) can cause more-severe illness, including epidemics of respiratory illness in settings such as military training camps [23, 24]. Adenovirus infections are spread by aerosol droplets, and risk factors for transmission of infection among military recruits include crowded conditions and smoking [25]. In the URECA population, it is interesting that the highest rates of adenovirus infections were found in the participants from New York City, which also has the highest population density among our sites [26].

Both the URECA and COAST studies are designed to identify environmental factors, including infections with respiratory pathogens, that influence the subsequent risk for developing asthma and respiratory allergy [1, 8]. The observed differences in the etiology of respiratory illnesses could influence the patterns of acute illness and could also affect long-term outcomes. For example, wheezing illnesses in infancy are an indicator of increased asthma risk, and this risk is further modified by the type of virus causing the wheezing. Adenovirus is of particular interest relative to long-term outcomes because acute infections can lead to viral shedding for weeks or months [22]. Since both the immune system and the lungs are rapidly developing in infancy [27], chronic infections could influence these developmental processes, leading to long-term changes in lung and/or immune function. Relationships between infections with specific viruses (including adenoviruses) in infancy and incident asthma will be analyzed in the URECA study when the children are 7 years of age.

This study has several strengths and some limitations that should be considered in interpreting its findings. Strengths of the study include the prospective collection of specimens during periods of illness and health, comprehensive viral diagnostic testing, and high rates of retention. In addition, the URECA cohort focuses on urban children of low socioeconomic status who have high rates of respiratory morbidity in early life and of asthma in childhood. Comparison to the COAST cohort is facilitated by the use of similar illness ascertainment and sampling methods and by use of the same viral diagnostic system [12]. The COAST and URECA studies both enrolled children with a family history of allergies or asthma, and there could be differences between patterns of respiratory illness in these children and patterns in the general population. The demographic characteristics of the cohorts are quite different, and since there are major differences in socioeconomic status, ethnicity, and lifestyle (Table 1), one limitation of the study is that multivariate analysis to determine which of these specific factors contribute to the different patterns of respiratory illnesses and pathogens is not possible. An additional limitation is that the samples from the 2 cohorts were collected 7 years apart. Since the samples collected in Madison yielded differences with all 4 of the urban centers and because continued monitoring in the COAST cohort has revealed persistently low rates of adenovirus isolation ([3] and unpublished data), the temporal difference is unlikely to account for the observed differences.

In summary, we compared the viral etiology of respiratory infection and illness in urban and suburban cohorts and found significant differences in the percentage of illnesses that were attributed to viral infection, as well as the patterns of specific viruses that were detected. These findings could influence acute and chronic respiratory health, and within the ongoing URECA study, the children will be carefully monitored for effects on asthma until the age of 7 years. These findings also raise additional questions about the etiology of respiratory symptoms in urban infants, particularly with respect to the cause of the respiratory illnesses for which no viruses were detected. Additional study of these samples by use of new broad-based genomic analysis techniques could provide further insight into this important area of unmet medical need.

### Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

### Notes

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