Viral Etiology of Frequently Recurring Respiratory Tract Infections in Children

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The viral etiology of frequently recurring respiratory tract infection (FRRI) in children aged <2 years was studied. Altogether, 329 children were followed from 2 to 24 months of age in the Finnish Otitis Media Cohort Study. Children with FRRI were defined as having ≥9 episodes of upper respiratory tract infection (URI) and/or ≥4 episodes of acute otitis media during follow-up. Nasopharyngeal aspirates, middle ear fluid specimens, and serum samples were analyzed for 8 common respiratory viruses. Of 1358 URI episodes, 642 (47%) occurred in the 78 children with FRRI. At least 1 virus was associated with 62% of these episodes, whereas the corresponding figure for children without FRRI was 54%. The frequency of different viruses was similar in both groups, but the relative proportion of rhinovirus infections was slightly higher among children with FRRI.

In conclusion, a specific viral etiology does not explain the excess of URI episodes in children with FRRI.

Upper respiratory tract infections (URIs) are a common problem in small children. Some children experience URI more frequently than do others or seem to have almost continuous symptoms of respiratory infections. Socioeconomic and environmental risk factors, such as day care attendance outside the home, number of siblings, a relatively short duration of breast-feeding, parental smoking in the household, and a polluted environment have been associated with frequently recurring respiratory tract infections (FRRI) [1–5]. FRRI have also been reported in children from families with general health problems [6]. In some studies, working mothers who have arranged for child care to be provided outside the home have been found to be more likely than other mothers to seek medical care for minor symptoms that could also be managed with family-initiated care [7]. Some biological factors, such as primary ciliary dyskinesia or gastroesophageal reflux, are rare causes of FRRI [8, 9]. Major defects in immune responses, which may bring about a chain of recurring infections, are rarely demonstrated in these children. Three prospective studies concluded that a tendency to acquire FRRI might be the result of a genetic risk factor, at least in some children who are assessed [10–12]. Although the leukocytes of children with FRRI have been reported to show deficient IFN-α production in vitro [13, 14], no unifying theory exists for this tendency to acquire recurring infections.

The putative inherited tendency to acquire FRRI could also reflect specific susceptibility to a certain type or group of viruses—for instance, through variations in the affinity of virus receptors in relevant tissues. In the Finnish Otitis Media (FinOM) Cohort Study, 329 children were closely followed from 2 to 24 months of age in an evaluation of respiratory infections and their etiology. The study design allowed us to compare the children with FRRI with the other children. In this study, we were especially interested in finding out whether the children with FRRI were...
particularly prone to infections caused by a specific virus or group of viruses.

STUDY COHORT AND METHODS

Study cohort and clinical follow-up. The study population consisted of 329 healthy children who were enrolled in the FinOM Cohort Study at the time of their second routine visit, at 2 months of age, to the well-baby clinic in Hervanta, Tampere, Finland. From April 1994 through July 1997, a study clinic with 1–2 study physicians and 2–3 study nurses was established. The study clinic was open in the daytime during the week and also for 3 h per day during weekends. The children were followed at the study clinic from 2–24 months of age. Blood samples were obtained from the children during visits scheduled to occur at 6, 12, 18, and 24 months of age. Data from interviews with the children’s parents were collected during scheduled visits at 2, 3, 4, 5, 6, 9, 12, 15, 18, and 24 months of age. The data obtained during these interviews included information on the children’s possible attendance at a day care center at the time of the visit. For the purpose of the present analysis, the date of the visit that occurred before day care center attendance was first recorded was registered as the day that a child began attending day care.

Altogether, 329 children (171 girls [52%] and 158 boys [48%]) were enrolled in the study. Forty-eight children did not complete the study. The reasons given for not completing the study were as follows: family moved to a new location (19 children) and withdrawal of parental consent (17 children). The parents of 11 children could not be contacted, and, for 1 child, no explanation was recorded in the database. The median age of the children at the time of study dropout was 15 months (range, 3–20 months). Overall compliance was good: 318 (97%) of the children remained in the study at age 6 months, 310 (94%) remained at age 12 months, and 294 (89%) remained at age 18 months [15]. A total of 281 children (85%) completed follow-up [15]. Data for all enrolled children are used in this study.

Each family was encouraged to take their child to the study clinic whenever he or she had symptoms of acute URI and, especially, if acute otitis media (AOM) was suspected. During these patient-initiated visits, patient history was recorded and a physical examination, including pneumatic otoscopy, was performed by the study physician. A nasopharyngeal aspirate (NPA) was routinely obtained by use of a sterile Pediatric Mucus Extractor (Orion Diagnostica). If AOM was diagnosed, myringotomy was performed and specimens of middle ear fluid (MEF) were obtained from the inflamed ear or ears. In addition, an acute-phase serum sample (“serum sample 1”) was obtained. The children were assessed at the study clinic ~4 weeks (14–42 days accepted) after AOM was diagnosed, so that resolution of the disease could be studied. At the time of this assessment, a convalescent-phase serum sample was obtained (“serum sample 2”).

Written informed consent was obtained from the parents of the children in the study. The study protocol was approved by the ethical committee of the National Public Health Institute (Helsinki, Finland) and by the ethical committees of Tampere Health Center and Tampere University Hospital (Tampere, Finland).

Definitions. URI was diagnosed if a child had symptoms and/or signs of an acute respiratory tract infection (e.g., rhinorrhea, fever, cough) and if the study physician diagnosed acute respiratory infection. For cases of URI, an NPA was obtained for evaluation. “AOM” was defined by a visually abnormal tympanic membrane (with regard to color, position, and/or mobility) that suggested MEF plus at least 1 of the following signs or symptoms of acute infection: fever, earache, irritability, diarrhea, vomiting, acute otorrhea not caused by otitis externa, or other simultaneously occurring respiratory tract symptoms [16].

A “confirmed AOM event” was defined as an occasion when AOM was diagnosed and middle ear effusion was demonstrated by collection of at least 1 MEF sample. Because inclusion of questionable cases of AOM could have biased the results, only confirmed AOM events were included in the analysis. Thus, 89% of all AOM events were included in the study.

An “AOM episode” was defined as a 30-day period that began with a diagnosis of AOM and included possible patient-initiated visits during this period. In accordance with this definition, a “URI episode” was defined as a period of 30 days that began when it was recorded, during any clinic visit, that a child had URI and/or AOM and that an NPA and/or an MEF sample was obtained. URI episodes thus could entirely or partially overlap with AOM episodes. A new episode could start when ≥30 days had elapsed since the beginning of the previous episode.

A “URI episode with coinciding virus infection” was defined by detection of a specific virus in an NPA, an MEF sample, or both, and/or serological documentation of viral infection at the onset of the URI episode (serum sample 1 collected on days −7 to +2 with respect to the onset of symptoms). A “virus-specific infection episode” was defined as a period of 30 days that began with the finding of an NPA and/or an MEF specimen positive for a given virus by any detection method and/or the observation of a significant increase in the concentration of antibodies to a given virus between serum samples 1 and 2. Detection of the same virus in the child during the next 30 days was considered to denote the same episode, whereas the presence of a different virus was considered to start an independent, overlapping episode.

For the purpose of the current study, the subjects were di-
vided into subgroups according to the number of reported infections. The 78 children (24%) who had experienced ≥9 URI episodes and/or ≥4 AOM episodes during follow-up were referred to as children with FRRI (from Pitkaranta et al. [11]). The remaining children were referred to as children without FRRI.

**Virus diagnostics.** The NPA and MEF samples were frozen immediately after collection and were stored at −70°C for 1–3 years before analysis. A time-resolved fluoroimmunoassay [17] was used to detect antigens for the adenovirus group, respiratory syncytial virus (RSV), parainfluenza virus types 1–3, and influenza viruses A and B in the NPA and MEF samples. The samples were analyzed for human rhinovirus group by use of a combined isolation–reverse transcriptase (RT) PCR method described elsewhere [18]. The samples were first cultured in HeLa Ohio cells in microtiter plates, and, thereafter, the cell culture suspensions were analyzed by RT-PCR. The sensitivity of the combined cell culture RT-PCR was 32% lower than that of a subsequently developed direct RT-PCR method [18].

Serum samples were frozen immediately after collection and were stored at −20°C. A standard micromethod for the CF test was used for the detection of virus antibodies to adenoviruses, parainfluenza virus types 1–3, influenza viruses A and B, Coxsackie virus B-5, and rhinoviruses [19]. A 4-fold increase between the concentration noted in serum sample 1 and that noted in serum sample 2 was considered significant. ELISA was used to detect antibodies for human coronaviruses OC43 and 229E by means of a previously described method [20]. A 2-fold increase in specific absorbances of paired serum samples was found to be significant. The IgG antibodies against RSV were measured with an ELISA that involved 1 dilution, by use of reagents and microplates from Virion (Microbe Scobe AG), except for the alkaline phosphatase–conjugated goat anti-human IgG (Caltag). The ELISA procedure was performed on serum dilutions of 1:100, according to the instructions provided with the Virion reagents, and the results were interpreted as described by Steinhoff et al. [21]. An increase in antibodies was significant if either the ratio of the optical density (OD) of the convalescent-phase serum sample to the OD of the acute-phase serum sample or the so-called E ratio of Steinhoff et al. [21] was >1.3, ruling out methodological error with 95% certainty.

**Data analysis.** Relative frequencies of demographic and clinical characteristics of children with FRRI and children without FRRI were compared by use of the χ² test. The odds of virus-specific URI occurring among children without FRRI with at least 1 URI episode, compared with that among children with FRRI, were compared by logistic regression analysis, with the grouping variable used as the only covariant in the model.

**RESULTS**

**Outcomes for children.** During the study period, 287 of 329 children had 1 or more patient-initiated visits, and, altogether, they experienced a total of 1358 URI episodes and 585 AOM episodes. For 42 children (13%), no URI episodes were recorded at the study clinic. The variation in the rate of both URI and AOM episodes among the remaining children can be...
Table 1. Demographic and clinical data for 329 children followed from 2 to 24 months of age in the Finnish Otitis Media Cohort Study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) of patients</th>
<th>With FRRIa (n = 78)</th>
<th>Without FRRIb (n = 251)</th>
<th>Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>.068</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>33 (42)</td>
<td>138 (55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>45 (58)</td>
<td>113 (45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of siblings</td>
<td>.015</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>29 (37)</td>
<td>133 (53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>27 (35)</td>
<td>79 (31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2d</td>
<td>22 (28)</td>
<td>39 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of day care</td>
<td>.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home care</td>
<td>41 (53)</td>
<td>166 (66)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family day care</td>
<td>6 (8)</td>
<td>26 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day care center</td>
<td>30 (38)</td>
<td>52 (21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1 (1)</td>
<td>7 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Had ≥2 siblings and/or attended day care</td>
<td>49 (63)</td>
<td>78 (31)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Adenotomy</td>
<td>49 (63)</td>
<td>17 (7)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Tympanostomy tube insertion</td>
<td>57 (73)</td>
<td>18 (7)</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. FRRI, frequently recurring respiratory tract infection.

a Defined as ≥9 upper respiratory tract infection (URI) episodes and/or ≥4 acute otitis media (AOM) episodes (n = 78).

b Defined as ≤8 URI episodes and/or ≤3 AOM episodes (n = 251).

c Statistical significance of the difference assessed by χ² test.

d Range, 2–6.

seen as a single continuum without apparent segregation into subgroups (figure 1). On the basis of the definition of Pitkäranta et al. [11], children with FRRI experienced 4–13 URI episodes (median, 8 episodes) and 1–10 AOM episodes (median, 4 episodes), whereas children without FRRI experienced 0–8 URI episodes (median, 3 episodes) and 0–3 AOM episodes (median, 0 episodes) during the study period.

The 78 children with FRRI comprised 24% of the study group and included more boys (58%) than did the group of children without FRRI (45%), but the difference was not statistically significant (P = .068). The proportion of children who had ≥2 siblings or who attended day care centers was larger in the group with FRRI than in the group without FRRI (table 1). In particular, if children had ≥2 siblings and/or attended a day care center, they had a significantly greater probability (P<.001) of having FRRI than did children who had no more than 1 sibling and who never attended a day care center.

Two-thirds of the children received care at home during the whole study period. Eventually, by the end of follow-up, a total of 82 children (25%) had attended a day care center; of these, 30 were children with FRRI and 52 were children without FRRI. For children who attended day care centers, the median number of URI episodes during follow-up was 6 (9 for children with FRRI and 3 for children without FRRI). For children who received care at home during follow-up, the median number of URI episodes was 3 (7 for children with FRRI and 2 for children without FRRI). The median age at the time of the first respiratory infection was 146 days for children with FRRI and 217 days for children without FRRI.

There was no statistically significant difference between the proportion of children with FRRI and that of children without FRRI who had such socioeconomic and environmental risk factors as premature birth, duration of breast-feeding, maternal smoking during pregnancy, parental smoking in the household, parental education, or being the child of a single parent (data not shown). Only 4 children (1%) were not breast-fed at all, and 51% of the children were breast-fed for >24 weeks. In addition, only 12 families (4%) reported that smoking occurred indoors in the home at any time during follow-up. As expected, significantly more children with FRRI had adenotomy (63%) and/or tympanostomy tube insertions performed during the follow-up period (73%), compared with children without FRRI (6% and 7%, respectively).

Viral etiology of FRRI. Designated URI episodes were used to assess the possible susceptibility of children with FRRI to specific viral infections. As a group, the 78 children with FRRI had 642 URI episodes; of these episodes, 401 (62%) were associated with an identified virus. For comparison, the 209 children without FRRI who had at least 1 URI episode had 716 URI episodes, of which 386 (54%) were associated with an
Table 2. Association of specific viral infections with episodes of upper respiratory tract infection (URI) in children with frequently recurring respiratory tract infection (FRRI) and in children without FRRI with at least 1 episode of URI.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Rate of incidence of episodes of URI per person-year among children</th>
<th>No. (%) of URI episodes among children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With FRRI <em>(n = 78)</em></td>
<td>Without FRRI <em>(n = 209)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>2.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Parainfluenza virus 3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Influenza virus A</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Other virusb</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>&gt;1 Virus per URI episode</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Virus-positive URI episodes</td>
<td>2.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Total URI episodes</td>
<td>4.5</td>
<td>1.7</td>
</tr>
</tbody>
</table>

a Percentage of all URI episodes in the respective group of children.
b Including coronaviruses 229E and OC43, Coxackie B-5 virus, influenza virus B, and parainfluenza viruses 1 and 2.

identified virus. The rates of incidence of URI episodes were 4.5 per person-year in children with FRRI and 1.7 per person-year in children without FRRI. The rates of incidence of URI episodes associated with specific viral infections per person-year (in particular, the rate of rhinovirus-positive episodes) were higher for children with FRRI than for children without FRRI (table 2). A total of 787 (58%) of 1358 URI episodes were positive for at least 1 virus: 568 (72% of all virus-positive URI episodes) were positive for rhinovirus; 147 (19% of all virus-positive URI episodes) were positive for RSV; 66 (8% of all virus-positive URI episodes) were positive for parainfluenza virus 3; 63 (8% of all virus-positive URI episodes) were positive for adenovirus; and 119 were positive for the other viruses for which testing was done. Children with FRRI had a slightly greater tendency to have URI episodes for which tests were positive for rhinoviruses, compared with children without FRRI (OR, 1.58; 95% CI, 1.27–1.96). The proportions of other different viruses associated with URI episodes were similar in children with FRRI and children without FRRI (table 2).

If the recurring infections were delineated by a positive viral diagnosis as a starting point (a virus-specific infection episode), a slightly different pattern of results was observed. However, the proportions of the different virus-specific episodes were again very similar in children with FRRI and children without FRRI (data not shown).

To confirm that the very frequent referral of children to the study clinic was also for genuine viral infections, we analyzed the virus positivity of URI episodes according to the number of URI episodes per child during the follow-up period. The overall virus positivity was similar (46%–68% [median, 56%]) in all categories, which suggests that viral infections had an equal role in the etiology of URI, irrespective of the frequency of URI. Furthermore, when children with ≥9 episodes of URI *(n = 35)* were analyzed individually for determination of rates of specific viruses found in association with the URI episodes, the distribution of different viral infections did not differ from that for the entire FRRI group (data not shown).

DISCUSSION

We have shown in this study that infection-prone children appear to acquire any viral infection available in the community, although a trend toward more frequent infections with rhinoviruses was observed. Rhinoviruses were the most frequently identified agents that caused URI in children with or without FRRI. A possible bias is that rhinoviruses were detected by a combination of viral culture and subsequent RT-PCR, which may be a more sensitive method than the antigen detection method used for the other viruses. Another bias is that serologic diagnosis was attempted only at the time of the patient-initiated visit for AOM. However, only a minor proportion of identifications of viruses as causative agents were made by serologic testing, and, in previous studies, it has been shown that the viral etiology of AOM is similar to that of URI [22].

Definition of FRRI is not easy, and different definitions can be found in the literature. We decided to use a pragmatic approach and selected single thresholds in the rates of designated URI and AOM episodes [11]. The designated group of children with FRRI appeared to have the desired characteristics, including a relatively greater number of siblings and attendance at day care centers. We found that as many as 48% of all docu-
mented URI episodes occurred in 24% of children who were included in the FRRI group. Analysis of the variation in the rate of URI development revealed a continuum without segregation of development among “infection-prone” and “normal” children; this suggests that the predetermined division into 2 groups may not be justified. It is unlikely, however, that an alternative approach—for example, use of continuous variables—would have changed the conclusion.

There was no difference between children with FRRI and children without FRRI with regard to some previously reported risk factors, including the duration of breast-feeding and parental smoking in the household. However, it was notable that very few families reported smoking indoors and that breast-feeding lasted $\geq 12$ weeks in more than two-thirds of the families [23]. Indeed, it has been suggested that fully breast-feeding (with the child not receiving any other type of milk) reduces acute respiratory infections during the first 4 months of life. Thereafter, the reduction is not significant [24]. In addition, in a recent study, breast-feeding was not found to reduce respiratory infections in an industrialized country [25]; however, it is considered an important factor in preventing infections and infant mortality in developing countries [26].

Structured investigation of FRRI is demanding because the individual variation in the length and intensity of the symptoms sometimes makes it difficult to judge whether a previous infection ceased before a new one commenced. We decided to use a standard 30-day length for episodes of AOM, URI, and virus-specific infection, according to FinOM Cohort Study studies [22]. We analyzed the etiology of infections in 2 ways with use of clinical URI diagnosis and virological findings as respective starting points. Both approaches yielded highly similar distributions of different viruses in the FRRI and non-FRRI groups. Furthermore, if the children were divided into an “infection-prone” quartile and a group of control children, on the basis of the number of patient-initiated visits per study month, the distribution of different viral infections was highly similar in the 2 groups (data not shown).

Although families were encouraged to take their child to the study clinic whenever he or she experienced acute URI, the number of URI episodes recorded in this study was smaller than that found in some previous reports [27]. Kilpi et al. [15] reported that 86% of all patient-initiated visits for AOM in our FinOM Cohort Study were captured at the study clinic, but it is possible that children who experienced mild symptoms were not always referred to the study clinic. It is not known whether the health care-seeking behavior varied between the families. However, because the overall virus positivity of URI did not vary significantly according to the number of reported URI episodes per child, this putative variation is unlikely to bias the results.

In conclusion, our results suggest that a specific susceptibility to a defined type or group of viruses does not seem to be a major explanatory factor for FRRI in small children. Rather, these children appear to acquire any infection circulating in the community.

Acknowledgments

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