Etiology of Measles- and Rubella-like Illnesses in Measles, Mumps, and Rubella–Vaccinated Children

Irja Davidkin, Martti Valle, Heikki Peltola, Tapani Hovi, Mikko Paunio, Merja Roivainen, Kimmo Linnavuori, Sari Jokinen, and Pauli Leinikki

The viral etiology of measles- or rubella-like illnesses after MMR (measles, mumps, and rubella) vaccination was studied prospectively in 993 acutely ill Finnish children with fever and rash in 1983–1995. Their sera were tested for adeno-, entero-, and parvovirus B19 antibodies. Sera of 300 children <4 years old were also tested for human herpesvirus 6 (HHV-6) antibodies. Measles and rubella had been excluded by previous antibody testing. Serologic diagnosis of adeno-, entero-, or parvovirus infection was based on EIA (IgM or IgG antibodies) and that of HHV-6 on indirect immunofluorescence. A viral etiology was verified in 368 cases, most commonly parvovirus (20%), followed by enterovirus (9%) and adenovirus (4%). Among young children, HHV-6 infection was found in 37 (12%). Thirty-eight children (4%) had double infections. This study confirms that measles- or rubella-like illnesses in MMR-vaccinated children are often caused by other viruses. Each suspected vaccine failure requires laboratory confirmation to maintain reliable surveillance and control and to establish the specific etiology of the disease.

In Finland, measles, mumps, and rubella are notifiable diseases. During the MMR (measles, mumps, rubella) vaccination program, started in 1982, an intensified follow-up of measles, mumps, and rubella was done [1]. From 1987, every suspected measles, mumps, or rubella case, including those in unvaccinated persons, had to be confirmed by specific virologic laboratory tests before notification. Although measles, mumps, and rubella became very rare in this country [2, 3], clinically suspected cases continue to occur. In most cases, they are presumably caused by some other virus presenting similar clinical symptoms.

In notified cases of measles, Brown et al. [4] showed, by laboratory tests, that only 15% of vaccinees and 47% of non-vaccinees really had measles, whereas Shirley et al. [5] confirmed rubella in only 36% of clinically suspected cases among unvaccinated persons. Parvovirus and human herpesvirus 6 (HHV-6) infections are often clinically misdiagnosed as measles or rubella. Anderson et al. [6] found rubella in 24% and parvovirus infection in 8% of patients suspected of rubella, and Tait et al. [7] found acute HHV-6 infection in 87% of children with clinical diagnoses of measles or rubella.

Inaccuracy of clinical diagnoses was soon realized to be a problem in Finland, because after introduction of the MMR program, most suspected cases were not confirmed serologically as an MMR disease. Foreseeing this difficulty, we set up a prospective study to determine the true etiology of diseases that were notified as measles or rubella. In this study, we searched for the viruses most commonly causing measles- or rubelliform disease: parvo-, entero-, and adenovirus and, among small children, HHV-6 [8].

Materials and Methods

Set-up. A national campaign aimed at eliminating measles, mumps, and rubella was launched in 1982 [2]. In that context, a specific study was set up to explore the viral etiology of MMR-like diseases that suggested vaccination failure. Whenever a vaccinating public health nurse or a physician was informed of suspicious symptoms with or without fever >3 months after vaccination, the case was reported to the National Public Health Institute, which gave instructions about sample collection and dispatch. The system was described in detail to the medical personnel of child health centers in a series of seminars organized throughout the country before the campaign [1]. Preferably, 2 serum samples were to be mailed 1–3 weeks apart.

Case selection. By 1995, at least 1 serum sample had been obtained from 2299 children who were reported to have measles- or rubelliform diseases and had received MMR vaccination. Serologic analysis showed that 123 of them had measles and 12 had rubella, this being together 5.9% of the whole series.

In addition to these 135 cases, those lacking the second serum sample (n = 353) and those with specimens otherwise inadequate (e.g., volume too small, inadequate timing; n = 71) are excluded from this study. Of the remaining 1740 vaccinees, 100 samples per year, if available, were randomly picked for the analysis. The first
sample had been taken, on average, 4 (±6) days after the onset of illness; the second, 16 (±7) days later. About 25% of the patients were <3 years of age, 50% were 3–7 years, and 25% were ≥8 years. Fifty-one percent (508) were girls and 49% (485) were boys. HHV-6 tests were done on all children <4 years of age (n = 300). Sera were kept frozen at −20°C until this study. The total number of samples studied was 993 paired sera.

Parvovirus antibody tests. IgM antibodies to human parvovirus B19 were measured with a commercial immunoassay kit (Parvovirus B19 IgM EIA; Biotrin, Dublin) according to the manufacturer’s instructions.

Adenovirus antibody tests. Adenovirus infection was detected by an in-house IgG EIA technique. We used purified adenovirus (type 2) hexon protein [9] as antigen. The plates (Polysorp; Nunc, Roskilde, Denmark) were coated with the adenovirus hexon antigen at a dilution of 2.5 µg/mL overnight at room temperature, saturated with 0.1% bovine serum albumin for 30 min at room temperature, and washed three times with PBS + 0.1% Tween 20. The paired sera were tested at 1/1000 dilution, 100 µL/well (dilution buffer: PBS + 0.1% bovine serum albumin + 0.05% Tween 20). After 1 h at 37°C and washings, horseradish peroxidase-conjugated anti-human IgG (1/18,000 dilution; A-6029; Sigma, St. Louis) was added to the wells for 1 h at 37°C, and the plates were washed again. The substrate o-phenylenediamine (00-2003; Zymed, San Francisco), 1 mg/mL, 100 µL/well, was incubated for 25 min in the dark at room temperature. Finally, the reaction was stopped by adding 1 M HCl, 100 µL/well. The optical density was measured with a multispan photometer at 492 nm. A panel of positive and negative control samples was examined in parallel in each test. The titers were calculated in arbitrary EIA units derived from a standard curve based on a dilution series of positive standards run in every assay. A diagnosis of adenovirus was confirmed if a 4-fold rise in EIA units was observed between paired sera.

Enterovirus antibody tests. Enterovirus antibodies were measured by an EIA in plates (Polysorp) coated with a synthetic peptide derived from an immunodominant region of capsid protein VP1 known to be a common antigenic determinant for most enteroviruses [10]. The assay procedure, previously validated by concomitant virus isolations [11, 12], was modified to be the same as for the adenovirus EIA except that sera and horseradish peroxidase conjugate were diluted 1/100 and 1/8000, respectively. High- and low-positive and negative controls were tested in all plates in each assay. Diagnostic criteria for an enterovirus infection were set from a series of parallel dilutions of positive sera. A 2-fold rise in optical density (OD) values of paired sera was considered a significant change. If the OD value of the first sample was already high, ≥1.500, a change of 0.500 (three times the interassay variation) or more was considered diagnostic.

HHV-6 antibody tests. HHV-6 antibody assays were done by an indirect immunofluorescence test [13]. The reciprocal of the highest dilution of a serum sample showing fluorescence was regarded as its antibody titer. A rise in antibody titers of at least 4-fold confirmed the HHV-6 diagnosis. Positive and negative controls were included in each assay.

Statistics. A comparative study of the likelihood of parvovirus infections during high- and low-incidence years was made with the χ² test.

Results

Occurrence of different infections. Altogether, among 368 children (37%), an acute infection with parvo-, entero-, or adenovirus or among young children <4 years old, HHV-6 was diagnosed serologically (table 1).

Parvovirus was the most common infection during two epidemic peaks in 1985–1986 and 1992–1993. During these years, parvovirus diagnoses were made in 35% of the studied children; in nonepidemic years, the proportion varied from 10% to 26%. During those 6 years when the parvovirus incidence exceeded 2/100,000, the likelihood of parvovirus diagnosis was 28% in patients with suspected cases who had exanthema and fever, whereas the corresponding likelihood during other years was only 13%. Thus, a diagnosis of parvovirus was 2.1 times (95% confidence interval, 1.6–2.8) more likely in high-incidence years than in low-incidence years. Entero- and adenovirus and HHV-6 infections did not show significant year-to-year variation during the observation period.

A significant change in enterovirus antibody levels was seen in 88 cases (9%). An additional 176 (18%) had a high (OD value >2.0) level, possibly reflecting a recent enterovirus infection.

Forty-four children (4%) met the diagnostic criteria for adenovirus infection. In addition, 178 patients (18%) had high (>70 EIA units) adenovirus IgG antibody levels, possibly reflecting a recent infection.

Of the 300 children <4 years old, 37 (12%) had evidence of acute HHV-6 infection. High (titer >160) antibody levels were found in 28 children.

Double infections were detected in 38 patients (4%). The most common combinations were parvo- and enterovirus or parvo- and adenovirus infections (13 and 9 cases, respectively).

Age-specific and seasonal trends. There was a clear age-dependence in the occurrence of the different viral infections. HHV-6 diagnoses peaked at 2 years, with 7 cases detected in 1- to 2-year-olds, 24 in 2-year-olds, and 6 in 3-year-olds. Entero- and adenovirus infections were more evenly distributed in the different age groups (figure 1A). Parvovirus infections were detected mostly among the 4- to 14-year-old children (figure 1A).

Table 1. Viral antibody findings among patients with suspected cases of measles and rubella.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Fulfilling diagnostic criteria (%)</th>
<th>High antibody levels (%)</th>
<th>Low antibody levels (%)</th>
<th>Seronegative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parvovirus</td>
<td>99 (20)</td>
<td>–</td>
<td>–</td>
<td>794 (80)*</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>88 (9)</td>
<td>176 (18)</td>
<td>552 (55)</td>
<td>176 (18)</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>44 (4)</td>
<td>178 (18)</td>
<td>586 (59)</td>
<td>185 (19)</td>
</tr>
<tr>
<td>HHV-6b</td>
<td>37 (12)</td>
<td>28 (9)</td>
<td>187 (63)</td>
<td>48 (16)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%).

* Only IgM test used.

b 300 children <4 years were tested for human herpesvirus 6.
Parvovirus infections also showed a clear seasonal variation, being most frequent in winter and spring (January–May) and in fall (October–November; figure 1B). Enterovirus infections showed a higher frequency in summer and early fall (July–September), whereas adenovirus and HHV-6 infections were evenly distributed throughout the year (figure 1B).

Discussion

The current study shows that in a large proportion of MMR-vaccinated children, with symptoms resembling measles or rubella, the true viral etiology can be determined. In our series of 993 children, 37% had a significant serologic finding suggesting either parvo-, entero-, or adenovirus or HHV-6 infection.

The prospective study design helped us to establish the specific diagnosis in a high proportion of cases. Particularly in years when parvovirus infections were common, the proportion of diseases identified rose to 41%, whereas in low-incidence years it varied between 12% and 33%.

The rather strict diagnostic criteria that we applied may have excluded some cases in which either the antibody response was delayed or the first serum sample was collected too late for detection of a significant increase in antibody level. Furthermore, the selection of viruses for testing in this study did not cover all of the viruses known to cause a rash with fever, which
may have caused the disease in some of these children. Therefore, in all likelihood, the current figures probably underestimate the extent to which other viruses were responsible for the etiology of measles- or rubella-like diseases in a vaccinated population. Moreover, bacterial and rickettsial infection and food or drug allergies may cause skin symptoms mimicking viral infections. The study design did not allow us to estimate the incidence of subclinical infections caused by these viruses.

In 1982, an intensified follow-up of all measles, mumps, and rubella cases was initiated along with the introduction of the program [2]. During the first 4 years following the launching of the national MMR vaccination, and with no major outbreaks of measles or rubella in the country, measles was found in 0.8% and rubella in 1.2% of suspected vaccine failure cases [2]. In the current follow-up covering the years from 1983 to 1995, rubella was detected in 0.5% and measles in 5.3% of suspected cases. However, 85% of these cases were found during the last measles-epidemic years, 1988–1989 [2].

In our study, the pathogen most commonly diagnosed (20%) was parvovirus. This is a higher proportion than was reported by Brown et al. (4%) [4] or Shirley et al. (7%) [5]. During our study period, two distinct peaks of parvovirus infection were noted, which contributed to this high value.

Most cases of enterovirus infections were detected in preschool children and occurred in late summer and fall, a well-known feature of enterovirus epidemiology. Adenovirus infections caused some of the measles- and rubella-like illnesses and did not show any clear-cut annual or seasonal variation.

The IHV-6 cases followed the expected epidemiologic pattern, mostly affecting children 1–2 years of age [8]. We did not collect detailed clinical information about the cases and so cannot conclude whether the detected double infections presented any atypical clinical features.

In our earlier study [14], we noted that levels of measles-specific antibodies in MMR-vaccinated children seem to decline faster in the 1990s, suggesting that a lack of natural boosters may modify the protection induced by the vaccination. Even a theoretical possibility of reemergence of measles among vaccinated persons warrants active surveillance of all measles-like diseases in a population in which measles is no longer endemic. Such a surveillance should also include an attempt to verify the viral etiology of these diseases. The current study demonstrates that this can be achieved with fairly simple laboratory tests, provided that adequate paired serum samples are available. This, together with the emerging possibility of tracing the origin of measles cases by sequencing techniques [15], underlines the importance of close collaboration between clinical practitioners, virologic laboratories, and the authorities responsible for the decisions on vaccination policy.

Acknowledgments

We thank Matti Waris for providing adenovirus hexon protein and Raija Vainionpää for collaborating in the adenovirus EIA. We also thank Seija Salmi for performing the laboratory tests excellently.

References