Detection of secondary mumps vaccine failure by means of avidity testing for specific immunoglobulin G

Juan Carlos Sanz-Moreno a,*, Aurora Limia-Sánchez a, Luis García-Comas b,1, Mª Mar Mosquera-Gutiérrez c,2, Juan Emilio Echevarria-Mayo c,2, Ana Castellanos-Nadal c,2, Fernando de Ory-Manchón c,2

a Laboratorio Regional de Salud Pública, Instituto de Salud Pública de la Comunidad de Madrid, C/General Orío 15, 28006 Madrid, Spain
b Servicio de Epidemiología, Instituto de Salud Pública de la Comunidad de Madrid, C/Julian Camarillo 4b, 28037 Madrid, Spain
c Centro Nacional de Microbiología, Instituto de Salud Carlos III, Ctra Majadahonda-Pozuelo s/n, 28220 Majadahonda, Madrid, Spain

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Abstract

The aim of this study was to discriminate between primary and secondary vaccine failure in children with mumps using IgG avidity testing. Thirty-nine serum samples from children with mumps, confirmed by specific IgM, were studied. The patients were grouped according to their immunization status. The secondary immune response was defined by IgG with an avidity index >32%. A secondary response in infected children previously immunized was considered as a secondary vaccine failure. Vaccinated children presented higher IgG titers and IgG avidity than unvaccinated children. The proportion of secondary immune responses in unvaccinated patients was lower than that obtained in previously vaccinated infected patients. Avidity testing can be a useful tool to detect secondary vaccine failure in mumps.

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1. Introduction

Mumps is generally a mild viral disease, which causes inflammation of the salivary glands. However, on some occasions it produces severe complications such as orchitis [1], pancreatitis [2] or thyroiditis [3]. A generally common manifestation of the disease is aseptic meningitis, which can produce side effects such as hearing loss [4]. These and other complications can be prevented by administering a live attenuated vaccine. The combined measles–mumps–rubella (MMR) vaccine was widely introduced in Spain in 1981 as a single dose in infants at 15 months of age. In the Madrid region (Autonomous Community of Madrid) a second dose was recommended in 1996 for 11-year-old children [5], and in 1999 this age criterion was lowered to 4-year-olds [6]. MMR vaccine coverage has progressively increased, reaching 94.9% for the first dose and 89.7% for the second dose in the year 2000 [7]. However, in spite of population-wide administration of this vaccine, mumps epidemics continue to occur [8]. It is important to distinguish between primary vaccine failure (no seroconversion after vaccination) and secondary vaccine failure (loss of immunity after seroconversion) when evaluating systematic immunization programs. Determining IgG avidity can, therefore, serve as a useful additional measure for this purpose [9]. During a primary infection, low avidity predominates, whereas in a past infection and a recurrent one (natural or as a result of live virus vaccination), it is high avidity, which predominates. The aim of this study was to discriminate between primary and secondary vaccine failure in children with mumps viral infection using avidity testing for specific IgG.
2. Methods

The study covered 39 serum samples from children with acute mumps, confirmed by specific IgM determination. Samples were obtained in the Madrid Community between April 2000 and July 2002. The children where selected if they had a concomitant positive IgG response to mumps virus. Only recorded documentation confirming administration of the vaccine was considered as indicative of prior MMR immunization (no children with unknown vaccination status were studied).

Patients were grouped into three categories according to their immunization status.

i. Group A consisted of nine unvaccinated children. Seven cases were epidemiologically linked to other cases.

ii. Group B consisted of 22 children who had received one MMR dose, 18 of them were epidemiologically related with other cases.

iii. Group C consisted of eight children who had been given a second dose of mumps vaccine during an outbreak (post-exposure vaccination group). All eight patients received the Jeryl Lynn strain as a second dose and all developed mumps symptoms in less than one month after administration. All of them were epidemiologically linked to other cases, and in four out of five cases with oral fluid samples, the culture, which was carried out using a human lung cancer cell line (NCI-H292) [10], was positive. Isolates were genotyped by sequencing the SH gene.

Serological tests (IgM, IgG and IgG avidity) were run by EIA (Enzygnost; Dade Behring), using Behring ELISA Processor III. IgG avidity tests were performed in two different runs using reagents from the same batch (Enzygnost IgG batch 33685; avidity reagent batch 34100). The urea concentration used in the IgG avidity test was 4.5 mol/l. The time and temperature of incubation with urea was 3 min at 15–25 °C.

The IgG avidity index (AI) was determined following a previously described procedure [11]. Serum samples were tested in duplicate wells on an EIA plate. One was determined as recommended by the manufacturer, while the other included an additional washing with a urea derivative, once the antigen antibody reaction had been completed. In the urea untreated wells all samples gave absorbances below the upper limit of the ELISA reader. However, in samples with absorbances higher than 2.5 an arbitrary titer of 3500 was assigned. The AI was calculated as the absorbance percentage observed in the urea treated wells, compared with absorbance in the untreated wells. The reliability of the results was assessed by testing the AI inter-assay variability obtained for the positive control (coefficient of variation rate for the AI of the positive control: 1.3).

1. Secondary immune response against mumps was defined as the presence of IgG with an AI ≥32% (high avidity) [12].

2. Secondary vaccine failure [9] was established if a secondary immune response was detected in infected children with a history of previous immunization.

Median or mean values and intra quartile range (IQR; range between 25th and 75th quartile) were estimated. Differences in quantitative variables such as age; duration of clinical disease; months elapsed from administration of the first dose; and finally mumps-IgG titers were calculated in the three groups using the Mann-Whitney U-test. Confidence intervals of percentages were calculated using the exact binomial test. Categorical variables, such as secondary immune response, were compared using the two-tailed Fisher test. All estimates were made with a 95% level.

3. Results

A summary of the results is shown in the Table 1. Although the mean age of the unvaccinated group was higher, no statistically significant differences were seen in the mean age of the three groups of patients overall. No significant differences were observed between patients in any of the groups as regard the duration of the clinical disease (period in days elapsed between the beginning of the disease and the blood sample collection). Nor were any significant differences identified in the average number of months elapsed from the first dose in children immunized with one dose (group B) or in post-exposure vaccination children (group C). Results of avidity assays are shown in the Fig. 1. Infected children previously vaccinated with one dose presented higher median IgG titers and higher mean AI IgG than unvaccinated children (Table 1). Children in the post-exposure vaccination group presented higher median IgG titers (not statistically significant) and higher median AI IgG than children with no history of vaccination. The median IgG titers in patients previously vaccinated with one dose was significantly higher than that observed in post-exposure vaccination children.

Fig. 1. Distribution of AI results in unvaccinated patients (group A) and in those vaccinated with one or two doses (groups B and C, respectively).
Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>IgM+ cases unvaccinated (n=9)</th>
<th>IgM+ cases 1 dose (n=22)</th>
<th>IgM+ post-exposure vaccination cases (second dose; n=8)</th>
<th>Outbreak related cases</th>
<th>Viral isolation</th>
<th>Median age (IQR)</th>
<th>Median days of disease (IQR)</th>
<th>Median of IgG titer (IQR)</th>
<th>Median of AI (IQR)</th>
<th>Patients with AI ≥32% (CI95%)</th>
<th>Mean months since first dose (S.D.)</th>
<th>Mean months since second dose (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>3/7</td>
<td>8 (4.0)</td>
<td>3 (4.0)</td>
<td>7088 (11635)</td>
<td>22.0 (21.5)</td>
<td>22.2 (2.8–60.0)</td>
<td>54.01 (41.5)</td>
<td>0.45 (0.3)</td>
</tr>
<tr>
<td>Group B</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3/11</td>
<td>4 (5.2)</td>
<td>4 (5.0)</td>
<td>23022 (17913.5)</td>
<td>62.5 (21.7)</td>
<td>95.5 (77.2–99.9)</td>
<td>50.24 (39.4)</td>
<td>0.50 (0.3)</td>
</tr>
<tr>
<td>Group C</td>
<td>4</td>
<td>8</td>
<td>2</td>
<td>4</td>
<td>4/5</td>
<td>3.5 (5.5)</td>
<td>2.5 (1.7)</td>
<td>12805 (9044.7)</td>
<td>48.5 (25.5)</td>
<td>75.0 (34.9–96.8)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

No significant differences in secondary vaccine failure were seen amongst patients immunized with one dose or amongst post-exposure vaccination patients. In the four patients in the post-exposure vaccination group in whom virus had been recovered from saliva, the wild type gene sequence (two genotype D and two genotype H) was found.

4. Discussion

Although MMR vaccination coverage can be considered excellent in Spain, seroprevalence of antibodies against mumps virus is lower than for measles or rubella [13]. Moreover, in recent years outbreaks of this disease [14,15] have been reported, thus indicating a reemergence. This phenomenon has been related to the use of a vaccine containing the Rubini strain [16] which was administered in the Community of Madrid from November 1996 to May 1999 [8], and which has been shown to provide low immunogenicity [17]. However, other more efficacious strains, such as Jeryl Lynn, do not ensure complete protection [8,15].

Unlike other viral diseases, immunity against mumps virus can be incomplete and recurrent infections may occur [18]. Understanding the role of primary and secondary vaccine failure is important for the evaluation of the national mumps control programmes. In an outbreak setting, the detection of contacts with secondary vaccine failures could be useful for indicating revaccination. Mumps viral infection in vaccinated subjects is often characterized by the presence of very high IgG titers [19]; a pattern indicative of secondary immune response. A positive correlation between IgG concentration and avidity to mumps virus has been previously reported [20], and the results of this study concur with the fact that previously immunized patients present both higher levels of antibodies (expressed as the median of IgG titers) and higher avidity than those detected in unvaccinated patients. Two out of nine unvaccinated patients showed high AI in relation to the cut-off established. Although the mumps vaccine can be considered safe, post-vaccine outbreaks appear eventually [21], and primary or secondary vaccine failure after one dose may be the cause of the appearance of cases after administration of a second dose. The eight patients in this study who had received two doses of MMR vaccine were linked with mumps outbreaks, and had received the second dose (in all cases containing Jeryl Lynn) as a prophylactic measure within a month before onset of the disease. This may have a particular influence on the immune response, which is elicited by a mix of natural and vaccine exposure. In four of these patients mumps virus was recovered from oral fluids and the wild type gene was identified. Detection of high avidity specific IgG in vaccinated patients with positive cultures would appear to back evidence of lack of protection, even in cases where there was an immune response to the virus. In the other four cases, post-vaccination exposure cannot be excluded. There is possibility than these cases were not due to the vaccine but rather to coincidental infection with wild-type
mumps virus, a finding, which has been previously reported [22]. Keeping in mind that the second dose of the vaccine could not evoke an immunological response in such a short time, these cases may be considered as secondary vaccine failures of the first dose, probably due to a wild strain (as in the case of the four patients where genotyping showed a wild-type).

IgG avidity tests prove useful for confirming primary infections as well as for differentiating between primary and secondary infections [23]. Although IgG avidity determination has classically been used in pregnant women to diagnose infections associated with congenital malformations such as rubella, toxoplasmosis or cytomegalovirus (by detecting low AI), high AI has been proposed as an immunological marker of the viruses included in MMR vaccine [24]. Most studies on this subject have, however been focused on the measles virus [9,11,25,26]. Unlike other infectious diseases, simultaneous detection of IgM and high IgG avidity does not exclude acute infection due to mumps virus. In fact, this serological pattern has been found in patients from whom the virus has been isolated. On the other hand, in the case of mumps, the cut-off for discriminating between high and low mumps-specific IgG avidity itself alone be regarded as a marker of a primary infection. This investigation was funded by a grant from the Instituto de Salud Carlos III (ISCIII02/25).

Avidity testing has been well standardized for infections such as rubella, toxoplasmosis or cytomegalovirus, however in the case of mumps, the cut-off for discriminating between low and high avidity has not been clearly defined. In this study the cut-off taken to indicate high avidity was the one set by other authors for the same EIA assay [12]. Although this cut-off may be questionable, due to the lack of other similar studies providing a good high avidity indicator, the significant differences between median values of AI seen in vaccinated and unvaccinated patients suggest the presence of a secondary immune response in the former. This fact could be explained as a consequence of a booster effect, due to natural infection in previously vaccinated subjects. Differences in AI were not affected by the duration of the secondary infections [23]. Although IgG avidity testing for analysis of measles virus infection. Clin Diagn Lab Immunol 1998;5(6):799–803.

Avidity testing can, therefore, be a useful tool to detect secondary vaccine failure in mumps cases. Nonetheless, additional studies are required to define appropriate cut-offs, in order to differentiate between high and low mumps-specific IgG avidity.

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**References**


