

Immune response to the mumps component of the MMR vaccine in the routine of immunisation services in the Brazilian National Immunisation Program

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A non-controlled longitudinal study was conducted to evaluate the combined vaccine against measles, mumps and rubella (MMR) immunogenicity in 150 children vaccinated in the routine of three health units in the city of Rio de Janeiro, Brazil, 2008-2009, without other vaccines administered during the period from 30 days before to 30 days after vaccination. A previous study conducted in Brazil in 2007, in 1,769 children ranging from 12-15 months of age vaccinated against yellow fever and MMR simultaneously or at intervals of 30 days or more between doses, had shown low seroconversion for mumps regardless of the interval between administration of the two vaccines. The current study showed 89.5% (95% confidence interval: 83.3; 94.0) seroconversion rate for mumps. All children seroconverted for measles and rubella. After revaccination, high antibody titres and seroconversion rates were achieved against mumps. The results of this study and others suggest that two MMR doses confer optimal immunoresponses for all three antigens and the possible need for additional doses should be studied taking into account not only serological, but also epidemiological data, as there is no serological correlate of protection for mumps.

Key words: MMR vaccine - immunogenicity - mumps

The combined vaccine against measles, mumps and rubella (MMR) widely used since 2003 by the Brazilian National Immunisation Program (NIP) is a lyophilised combined preparation of strains of attenuated measles (Schwarz strain), mumps (RIT 4385 strain derived from Jeryl Lynn strain) and rubella (Wistar RA 27/3) viruses. Immunogenicity of the components measles and rubella has been universally excellent, but for the mumps component this evaluation has been a complicated matter, due to different sensitivities and accuracies of the assays. Even neutralisation assays may provide different results according to the challenging virus. There is no established serological correlate of protection for mumps (Rubin & Plotkin 2013) and so we evaluated immunogenicity by “seroconversion” or “seropositivity”, instead of seroprotection.

In a Brazilian study done by our group (Silva et al. 2011) in a cohort of children from about 12-15 months of age vaccinated against yellow fever and MMR simultaneously or at intervals of 30 days, low seroconversion

for mumps was observed, 61% and 71%, respectively. It should be noted that in that study vaccines from two different producers [GlaxoSmithKline (GSK) and Merck] were used in approximately the same proportion and generated similar results.

Those unexpected results, if confirmed, could indicate that a substantial proportion of children was not protected by the vaccine provided by the Brazilian NIP. We conducted a study to explore further the immunogenicity of the GSK MMR vaccine, given without other viral vaccines being administered 30 days before or after MMR vaccine.

SUBJECTS, MATERIALS AND METHODS

This is a non-controlled, longitudinal study, carried out in a sample of 150 children 12-15 months of age in three health units of the municipality of Rio de Janeiro who received the combined MMR vaccine according to the basic immunisation schedule and NIP routine procedures. Healthy male and female children, living in the city of Rio de Janeiro, with no significant past medical history, were selected for this study. The criteria for not including into the study were: (i) subjects with a history of measles, rubella and/or mumps, (ii) subjects who had already received MMR vaccination documented in the vaccination card, (iii) subjects with a history of receiving blood transfusion or blood products, including immunoglobulin in the past one year previous to the study, (iv) subjects who presented skin lesions at sites of venipuncture and (v) subjects who had used corticosteroids (except topical or aerosol) during the last six months or reported

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use of immunosuppressive drugs. Two blood samples were collected: before and 42 days after vaccination (minimum acceptable 30 days and maximum 60 days). Volunteers who did not seroconvert from seronegative to seropositive or who had inconclusive results for any of the antigens were vaccinated again and a third blood sample was collected within the same time range recommended for the second blood collection. Medical records were prepared using the Teleform Workgroup 2008 program, v.10.2, which allowed capture of the scanned data, without manual typing and creation of a database.

Vaccine used on the study - The MMR received in bulk from GSK, formulated and distributed by Bio-Manguinhos, Oswaldo Cruz Foundation (Fiocruz), single lot (072VVA007Z), in 10 dose presentation, February/2007 production date and valid for two years, was used on the study. Each 0.5 mL dose of reconstituted vaccine contained at least 1,000 50% cell culture infectious dose (CCID₅₀) of attenuated measles virus, Schwarz strain, at least 1,000 CCID₅₀ of attenuated rubella virus Wistar RA27/3 strain and at least 5,000 CCID₅₀ of attenuated mumps virus, RIT 4385 strain, derived from Jeryl Lynn strain. For each component of the vaccine batch used in the study, the potencies at temperatures varying from 2°C to 8°C were, in Log₁₀, 4.26 (measles), 5.28 (mumps) and 4.02 (rubella) and, after storage at 37°C, 3.96, 4.99 and 3.85, respectively. The vaccine administered into each volunteer was diluted at the time of study enrollment, according to the NIP procedures and each volunteer received only the first dose from each vial.

Laboratory methods - Blood samples were placed into an insulated box kept between 4-10°C from time of collection until arrival at the laboratory. The maximum time between blood collection and arrival at the laboratory was 6 h.

IgG antibodies against measles, mumps and rubella were determined at the Reference Laboratory for Measles and Rubella, Oswaldo Cruz Institute/Fiocruz using an enzyme immunoassay (EIA) with a commercial kit from Siemens (Enzygnost® IgG). The results for optical density were converted to international units or units per millilitre of serum using a table provided by the manufacturer and categorised as negative for rubella, mumps and measles if < 4.0 IU/mL, < 231 U/mL and < 150 mIU/mL, respectively. These cut-offs were used in all studies referred to in the bibliographical references. Additionally, results were categorised as seronegative, inconclusive and seropositive, according to the kit instructions. At the end of 2010, samples were sent for retesting for the mumps component at the GSK Biologicals laboratory (Rixensart, Belgium) using the same methodology (EIA) and diagnostic kit used in the reference laboratory at Fiocruz.

IgM antibodies against mumps were measured after revaccination of children seronegative after the first dose in order to detect primary immune failures.

Data analysis - A database for the study was created using the Statistical Package for Social Sciences program v.17. The distribution of absolute and relative frequencies of subjects were tabulated by sex, age group,

health units, interval between date of vaccination and date of blood sample collection. Immunogenicity of the MMR vaccine was assessed primarily in terms of the percentage of baseline seronegative children who seroconverted for antibodies against measles, mumps and rubella viruses (that is, developed antibody levels \geq cut-off for seropositivity after vaccination). Criteria for protocol adherence were: children seronegative before vaccination with available serologic test results before and after vaccination and blood collected from 30-60 days after vaccination. We constructed 95% confidence intervals (CI) for proportions using Winpepi (Abramson 2011). Immunogenicity was also evaluated by geometric mean titre (GMTs) after vaccination and the magnitude of the immune response could be assessed against the minimum antibody levels for seropositivity.

Ethical aspects - The MMR vaccine administered to volunteers was the same used in the NIP routine, having already gone through immunogenicity and reactogenicity clinical studies before registration and use on a large scale. The study protocol was approved by the Research Ethical Committee of the Municipal Health Secretariat of Rio de Janeiro (protocol 48/08). All procedures were performed after parents/tutors agreement and signed informed consent.

RESULTS

From May-August 2008, 165 children were enrolled, of which 150 were eligible and 146 (96.7%) had blood samples obtained before and after vaccination. There was a slight male predominance (55%) and most children (92.7%) were from 12-15 months of age. The intervals between vaccination and blood sampling after vaccination ranged from 34-73 days and of 146 volunteers who had a second blood sample collected, 97.9% had intervals from 30-60 days. Before vaccination, there was one child seropositive for measles, one for mumps and one for rubella and 143 children were susceptible to measles, mumps and rubella. After vaccination, seroconversion for measles and rubella was 100% for children initially seronegative.

According to the kit instructions and in children with adherence to protocol, 86 (60.1%) were seropositive for mumps, 42 (29.4%) inconclusive and 15 (10.5%) seronegative. For all children, with exception of one child seropositive to mumps before vaccination, 88 (60.7%) were seropositive and the number with negative or inconclusive results after vaccination was 57 (39.3%). Considering 231 U/mL as the cut-off, in children with adherence to protocol, seroconversion for mumps after first vaccination was 89.5% (95% CI: 83.3; 94.0) and 130/145, 89.7% (95% CI: 83.5; 94.1), for all available children who were seronegative before vaccination (Table I).

Post-vaccination GMTs were 2,234 (95% CI 2039.4; 2447.7) mIU/mL for measles, 596.6 (95% CI 517.2; 688.3) U/mL for mumps and 50.1 (45.1; 55.8) IU/mL for rubella. When contrasted with cut-off values for seropositivity, the GMTs indicated considerably larger magnitude of the immune response for rubella (cut-off: 4.0 IU/mL)

Neither health unit nor time of blood collection after vaccination was relevant regarding immunogenicity (data not shown).

Of the 58 children seronegative or with inconclusive serology for mumps after vaccination, 57 were eligible for revaccination (1 child was excluded due to a 2nd dose of MMR vaccine received during a MMR campaign, registered on the vaccination card). Blood samples were collected after the second vaccine dose in 54 children (94.7%) and were not collected in three children due to parent/tutor refusal. The interval between vaccination and revaccination ranged from 203-249 days, with a mean of 221 days [standard deviation (SD): 11.6] and a median of 220 days. The interval between revaccination and third blood collection ranged from 31-64 days, with a mean of 39 days (SD: 6.2) and a median of 37 days.

After revaccination, all children had high IgG titres for mumps, above 1,200 U/mL, with exception of one child, who had a titre of 457 U/mL. Measles and rubella antibody titres showed a modest rise (Table II).

Moreover, after revaccination, IgM for mumps was negative in 52/54 (96.3%) children and in two children results were inconclusive; for measles, 50/54 (92.6%) were IgM negative and four were inconclusive; for rubella, 53/54 (98.1%) were IgM negative and one was inconclusive.

Due to the high percentage of negative and inconclusive results for mumps after the first MMR vaccination, according to the kit instructions, the pre and post-vacci-

nation samples were sent for blind retesting for mumps at the GSK in Rixensart, Belgium. Agreement of results from test and retest was high: Weighted kappa = 0.96.

DISCUSSION

This study confirms that MMR immunogenicity is excellent for measles and rubella. However, mumps immunogenicity seems to be not so high after one dose. Mumps immunogenicity has been highly variable across MMR studies with features similar to the current study, that is, with the same Jeryl-Lynn based vaccine from GSK, after the first dose (MMR used alone or with simultaneous administration of varicella vaccines), with similar age at vaccination, using the same Enzygnost® kit and ≥ 231 U/mL cut-off for seropositivity. Of note, none of these studies used the kit criteria for mumps inconclusive results. All assumed ≥ 231 U/mL for mumps indicated seropositive results (Usonis et al. 1998, 1999, Crovari et al. 2000, Nolan et al. 2002, Goh et al. 2007, GSK 2010, Rümke et al. 2011, Silva et al. 2011). On these studies, GMTs ranged from 414.1-1,640.5 and seroconversions from 70.8-98.6%. Studies from 1999-2002 presented the highest immunogenicity. A study in 2011 in Germany showed a GMT for mumps of 523.7 U/mL, with 71.3% seroconversion (Rümke et al. 2011).

Efficacy trials represent the “best scenarios” of vaccine performance under controlled conditions and are commonly required before a new vaccine is licensed. They are measured usually as immune responses and when there are correlates of protection (that is, a cut-off of antibodies above which there will be protection against the disease), it is possible to estimate vaccine effectiveness from immunogenicity data. This is the case for measles and rubella. In the case of mumps, there are no correlates of protection, so it is not possible to estimate effectiveness - that is, the magnitude of reduction of disease rates attributable to vaccination under real life conditions. Many studies evaluated effectiveness of the mumps component in populations vaccinated with Jeryl-Lynn based vaccines. The recent mumps outbreak in New York and New Jersey reported by the Centers for Disease Control and Prevention (CDC 2010) estimated a variation on mumps vaccine effectiveness from 73-91%

TABLE I
Results of mumps serology (enzyme immunoassay) after the first dose of combined vaccine against measles, mumps and rubella

IgG serology	Cut-off 231 U/mL n (%)	According to kit n (%)
Negative	15 (10.5)	15 (10.5)
Inconclusive	-	42 (29.4)
Positive	128 (89.5)	86 (60.1)
Total	143 (100)	143 (100)

TABLE II
IgG (ELISA) geometric mean titres (GMT) after vaccination and revaccination and revaccination/vaccination ratios, for measles, mumps and rubella

IgG titres	n	After vaccination GMT (95% CI)	After revaccination GMT (95% CI)	GMT ratio revaccination/vaccination (95% CI)
Measles (mIU/mL)	54	2,155.5 (1,828.0; 2,541.6)	5,692.1 (4,815.5; 6,728.4)	2.6 (2.3; 3.1)
Mumps (U/mL)	54	247.6 (214.3; 286.0)	3,157.0 (2,684.9; 3,712.0)	12.8 (10.3; 15.8)
Rubella (UI/mL)	54	41.3 (33.8; 50.4)	151.0 (134.2; 169.9)	3.7 (3.0; 4.5)

after one dose and from 79-95% after two doses. Even so, overall vaccine effectiveness is not questioned, as the annual number of mumps cases in the United States of America decreased from 186,000 in 1967, when the vaccine was introduced, to less than 500, in the early 2000s. Waning immunity, that is, decrease on seroprotection since time of last vaccination, seems to be an important factor for vaccination failure, both for one and two-dose vaccinees. Waning may also partly explain why vaccination effectiveness has been in general lower than efficacy, which is usually assessed after shorter follow-up.

There is a trade-off between mumps vaccine immunogenicity and reactogenicity, mainly regarding aseptic meningitis. Clearly, the Jeryl Lynn based vaccines are the safest, although probably not the most immunogenic. This evaluation should be done by each country, according to epidemiological considerations and degree of tolerance for adverse events. It should be noted that Brazil has had a negative experience regarding adverse events with MMR campaigns using mumps strains other than Jeryl-Lynn (Dourado et al. 2000, da Cunha et al. 2002, Silveira et al. 2002).

The strong response to mumps revaccination on the current study, with a very high after revaccination/after vaccination GMT ratio, suggests insufficient power of this vaccine to induce strong mumps immune response after one dose, but the booster response is reassuring concerning seroconversion after two doses. Moreover, after revaccination, 96.3% of children were IgM negative for mumps, again suggesting a secondary immune response, with the caveat that the blood collection was taken from one-two months after revaccination, when IgM levels are expected to be on the decrease. However, high IgM levels after disease in unvaccinated subjects are maintained for several weeks or months (CDC 2012). Assessment of IgM levels after MMR first dose was found in only one bibliographical reference, using the Hoshino mumps strain, the ELISA IBL kit, with blood collected four-seven weeks after vaccination and IgM seropositivity was found in 71% of children (Tabatabaei 2013).

Limitations in the accuracy of the mumps laboratory test, particularly its sensitivity (81%), may have contributed to the suboptimal mumps immunogenicity results (Backhouse et al. 2006).

These considerations assumed no relevant virus circulation, which seemed reasonable, even though reporting of mumps cases is not mandatory in Brazil, except outbreaks, which are usually perceived in health care units and are reported on the national notification system, which was not the case.

The results of this study are in agreement with two previous studies done by our group, the first already referred (Silva et al. 2011), which included 1,769 children, and a second not yet published, but with final report approved, which included 183 children 12-18 months of age, using the same methodology and cut-offs.

In the absence of accepted correlates of protection for mumps, no definite statements regarding protection against disease can be derived from the results of this study. However, the data here provided strengthen the

need of a second MMR dose to ensure maximum protection against mumps. Serological and epidemiological studies after two doses should be implemented to know if further doses and at which intervals are needed.

Data from the current study confirm the high immunogenicity of the MMR measles and rubella components with one dose and suggest lower immunogenicity of the mumps component. After a second dose, all volunteers seroconverted to mumps, with high antibody levels. The main and immediate implication of these findings is the reassurance that two MMR doses are highly immunogenic for all antigens included on the vaccine. As there is no serological correlate of protection for mumps, the implications of immunogenicity data should be considered cautiously, but they may be useful regarding immunisation practices and guidance, taking into account other variables, such as epidemiological data.

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