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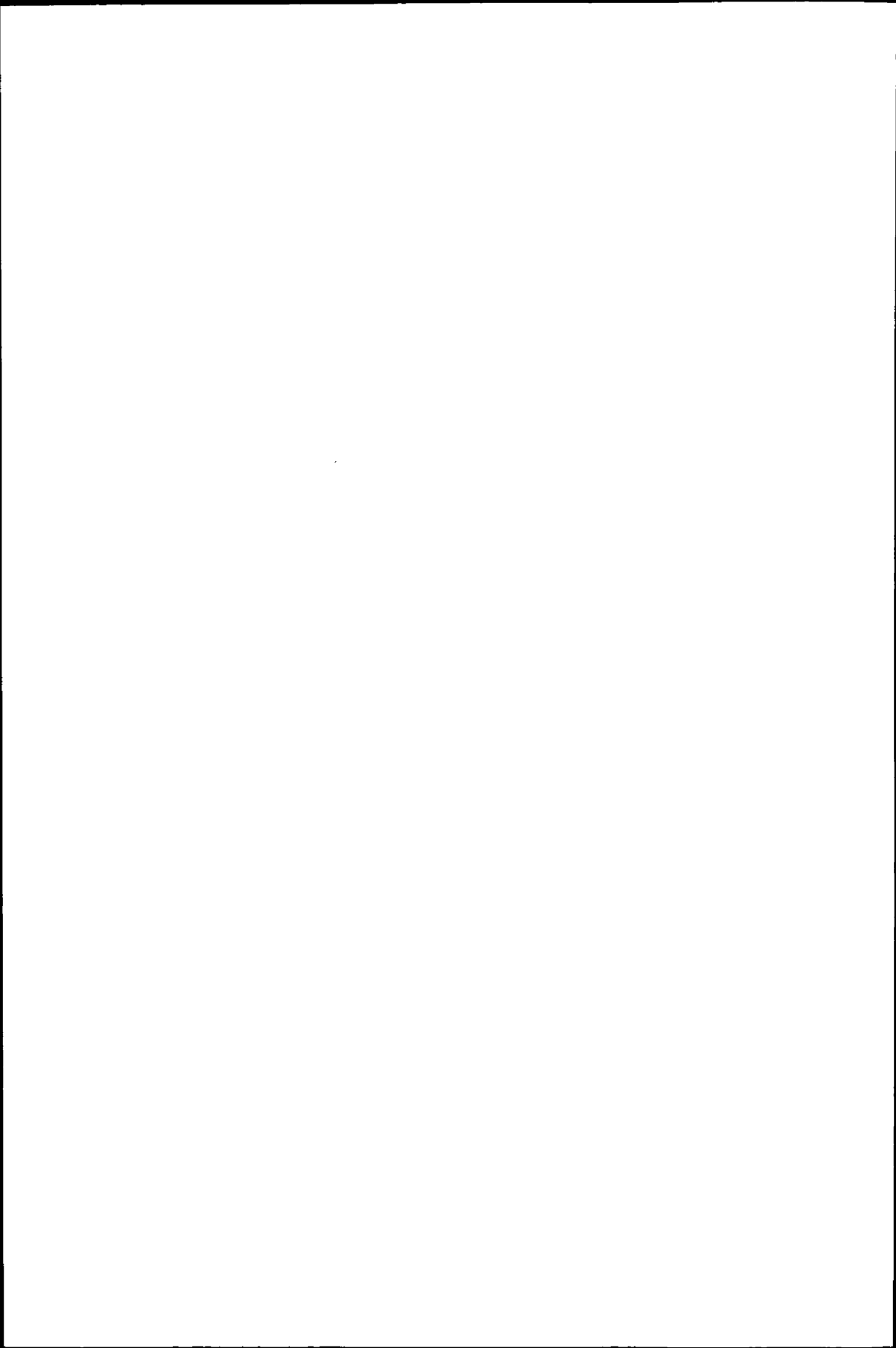
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Chapter 3

Immunogenicity of second dose measles-mumps-rubella vaccine and implications for serosurveillance



Immunogenicity of second dose measles–mumps–rubella (MMR) vaccine and implications for serosurveillance

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Abstract

Measles and mumps, but not rubella, outbreaks have been reported amongst populations highly vaccinated with a single dose of measles–mumps–rubella (MMR) vaccine. Repeated experience has shown that a two-dose regime of measles vaccine is required to eliminate measles. This paper reports the effect of the first and second MMR doses on specific antibody levels in a variety of populations.

2–4 years after receiving a first dose of MMR vaccine at age 12–18 months, it was found that a large proportion of pre-school children had measles (19.5%) and mumps (23.4%) IgG antibody below the putative level of protection. Only a small proportion (4.6%) had rubella antibody below the putative protective level. A total of 41% had negative or equivocal levels to one or more antigens. The proportion measles antibody negative (but not rubella or mumps) was significantly higher in children vaccinated at 12 months of age than at 13–17 months. There was no evidence for correlation of seropositivity to each antigen, other than that produced by a small excess of children (1%) negative to all three antigens. After a second dose of MMR, the proportion negative to one or more antigens dropped to <4%. Examination of national serosurveillance data, found that following an MR vaccine campaign in cohorts that previously received MMR, both measles and rubella antibody levels were initially boosted but declined to pre-vaccination levels within 3 years.

Our study supports the policy of administering a second dose of MMR vaccine to all children. However, continued monitoring of long-term population protection will be required and this study suggests that in highly vaccinated populations, total measles (and rubella) IgG antibody levels may not be an accurate reflection of protection. Further studies including qualitative measures, such as avidity, in different populations are merited and may contribute to the understanding of MMR population protection. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Rubella; Measles; Mumps

1. Introduction

Measles outbreaks in cohorts highly vaccinated with a single dose of MMR vaccine have been frequently reported, mumps outbreaks occasionally and rubella never [1–5]. Reduced vaccine effectiveness has been explained as due either to primary (PVF) or secondary vaccine failure (SVF). PVF represents a failure of immediate seroconversion with a documented lack of detectable-specific antibody. Administration of a second dose of vaccine to primary vaccine failures results in a high proportion undergoing a primary antibody response, with an initial IgM response followed by IgG seroconversion. SVF is infection in an individual following initial documented seroconversion, and represents

a loss of protection, often linked to waning serum antibody levels [6]. Following a second dose of vaccine, a large boost in IgG antibody levels generally occurs, with little or no IgM response. In the case of measles, although specific concentrations of IgG antibody have been postulated as protective thresholds against clinical disease [7], a graduated relationship between IgG level and degree of protection may be more likely.

For mumps vaccine, vaccine failure rates vary depending on the vaccine strain. PVF plays an important role, but the relative contribution of SVF is unclear [1,5,8,9]. For measles vaccine, the situation following a single vaccine dose is also unclear [9–13]. Clinical disease amongst vaccinated individuals exposed to wild measles virus is milder or asymptomatic compared to unvaccinated individuals [14,15] and suggests a role for SVF. However, the epidemiological significance of waning population immunity in relation to sustained transmission remains uncertain [16].

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Despite high first dose coverage, most countries in North America and Western Europe have introduced a routine second dose of MMR vaccine, to overcome the problems of vaccine failure [17,18]. In the UK, single dose MMR replaced single antigen measles at 12–15 months of age in 1988. In 1994, a national campaign vaccinated 92% of 5–16 years old with MR vaccine, to prevent a predicted measles epidemic [19]. In October 1996, a second, pre-school MMR dose was added to the immunisation programme. These changes resulted in a low reported incidence of measles, mumps and rubella in the UK in the 1990s.

This paper reports the effect of measles, mumps and rubella vaccination on specific antibody levels in a variety of populations of children in whom there has been little opportunity for natural boosting. The paper specifically explores the type and level of antibody response following the first and second dose of MMR vaccine and discusses the implications for immunisation strategy.

2. Methods

Serological data were acquired from two sources: a vaccine trial (for acellular pertussis vaccine in pre-school children [20]) and from the national serological surveillance programme in England and Wales [22].

2.1. Pre-school booster trial (PSB) for DTaP and MMR

This trial was a phase 2 acellular DTP (DTaP) vaccine booster trial in a population of pre-school children. Details of the double-blind randomised study design, population and ethical approval are reported elsewhere [20]. Due to the introduction in the national vaccination programme of a second dose of MMR midway, there was an opportunity to measure the measles, mumps and rubella IgM and IgG levels in populations who had not received a second dose.

The PSB study recruited a total of 1033 children aged 3.5–6 years between 1995 and 1997. Of these a sample of 610 eligible children were selected between September 1995 and October 1997. All had received a first dose of MMR. Before October 1992, three MMR vaccines were used: Pluserix (SmithKline Beecham, containing Schwarz measles strain, RA 27/3 rubella, Urabe-9 mumps strain); MMR II (Merck Sharp Dohme, containing Enders' Edmonston measles strain, RA 27/3 rubella, Jeryl Lynn mumps) and Immravax (Merieux, containing Schwarz measles strain, RA 27/3 rubella, Urabe-9 mumps strain). After 1992, only the MMR II vaccine was used. Children recruited to the study after October 1996 were offered a second dose of MMR II, although this was not a condition for enrolment. The vaccines were administered simultaneously by the study nurses by intramuscular injection in the arm, leg or buttock. MMR was administered at a different injection site to the DTaP or DT vaccine. Venous blood samples were collected from each child 4–6 weeks after the second MMR dose.

A total of 389 (63.7%) children were bled 4 weeks after receiving only a pre-school acellular DTP or just DT booster, and 221 (36.3%), 4 weeks after receiving a pre-school DTP/DT booster and MMR second dose. The sex distribution of the two groups was balanced (one dose group 48% female, two dose group 50% female, $P = 0.65$).

2.2. Serological surveillance data for rubella and measles

The PHLS serological surveillance programme involves the annual collection of residual sera from 18 public health laboratories throughout England and Wales. These are tested for a variety of antibodies including to measles, mumps and rubella. The sera are anonymised, only retaining information on age, sex and collecting laboratory [21].

The surveillance data were analysed to examine the second dose effect of the 1994 MR campaign on the 1988 and 1989 birth cohorts for the 4 years between 1994 and 1997. These birth cohorts would have been scheduled for a single dose of MMR vaccine at age 12–18 months, and to receive MR vaccine in the campaign in November 1994. The 1995–1997 results thus represent antibody levels in the 3 years after this second dose. For the 1988 birth cohort 96, 93, 90 and 103 sera were tested for the years 1994–1997, respectively, with 95, 92, 90 and 109 sera, respectively from the 1989 cohort.

2.2.1. Laboratory methods

Serum specimens from both trials and from the national serosurveillance programme were separated, stored at -30°C and transported frozen to Preston Public Health Laboratory where they were tested for IgG antibody specific for measles, mumps and rubella.

Commercial enzyme immunoassays were used. For measles, an ELISA kit (Gull Ltd.; distributed by Launch Diagnostics, catalogue number RGE-100) was used, including the Second British standard (diluted in PBS) as control serum. Quantitative IgG results were classified as positive, equivocal or negative according to the concentrations: ≥ 100 mIU/ml positive, 50–99 mIU/ml equivocal, and 0–49 mIU/ml negative.

For mumps, an ELISA kit (Human Ltd., Germany; UK distributor Biostat Ltd., catalogue number 851207) was used together with an in-house standard developed as part of the European Seroepidemiology Network project (ESEN) [23]. Quantitative IgG results were classified according to the kit cut-offs.

For rubella, an ELISA kit (Trinity Biotech, Eire; UK distributor Microgen Ltd., catalogue number 801–335) was used together with the WHO standard (diluted in PBS) as control serum. Quantitative IgG results were classified as positive, equivocal or negative according to the following concentrations: ≥ 10 IU/ml positive; 5–9 IU/ml equivocal and 0–4 IU/ml negative.

For the PSB study, sera were also sent to the Central Public Health Laboratory, London to be tested for IgM antibody specific for measles, mumps and rubella antibody-capture radioimmunoassay (MACRIA) was used as previously described [23]. IgM antibody positivity was defined as a test/negative (T/N) ratio >2.5 for rubella and measles and 3.0 for mumps. Repeat testing of all positives was undertaken by the same method. Sera were reclassified according to the same cut-off ratios. All laboratory testing was performed blinded without knowledge of the vaccination status of the individual.

2.2.2. Data entry and statistical analysis

The study was co-ordinated by the Immunisation Division of the Public Health Laboratory Service Communicable Disease Surveillance Centre. Information from the clinical record cards was entered at CDSC.

Statistical analysis was undertaken in Epi-info version 6.04 [24]. Antibody levels were log-transformed for calculation of geometric mean titres (GMT), concentrations (GMC) and 95% CI. Means were compared among vaccine groups using the Students' *t*-test. Differences between vaccine groups in proportions above specified levels and other group characteristics were tested by χ^2 and Fishers exact test as appropriate.

3. Results

3.1. Pre-school booster study

3.1.1. Measles

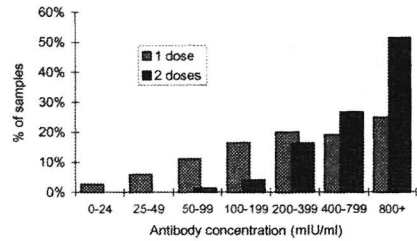
The distribution of IgG antibody in the two groups is shown in Fig. 1a. A significantly larger proportion (8.5 and 11.1%, respectively) of those who had received only a single dose of MMR vaccine were measles IgG antibody negative or equivocal, compared with 0 and 1.4%, respectively of those who had recently received a second dose of MMR. The geometric mean antibody concentration was significantly higher in the group who had received two doses of MMR (673.7 mIU/ml, 95% CI 616.6–741.3) compared with those who had received only a single dose (289.7 mIU/ml, 95% CI 257.0–323.6) ($P < 0.00001$). Of those who had received only a single dose, the proportion who were antibody negative or equivocal was significantly higher in children vaccinated at 12 months of age than in children vaccinated at 13–17 months of age (55/186 versus 16/151, $P < 0.0001$).

Only a small proportion of either group (2.1% of one dose, 0.9% of two dose children) had IgM measles antibody. There was no significant difference between the two groups (Yates corrected $\chi^2 = 0.55$, $P = 0.46$).

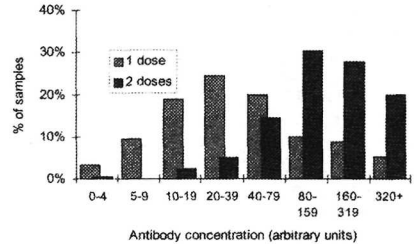
3.1.2. Mumps

The distribution of IgG antibody is shown in Fig. 1b. A significantly larger proportion of single-dose children were mumps IgG antibody negative or equivocal (14.9 and

a. Measles



b. Mumps



c. Rubella

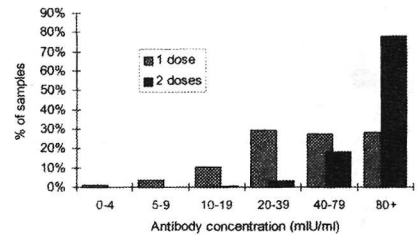


Fig. 1. (a-c) Measles, mumps and rubella IgG antibody distribution in PSB study. Comparing one dose group: 4 years post-dose 1 and two dose group: 6 weeks post-dose 2.

9.8%, respectively), compared with only 1.4 and 0.9%, respectively of those receiving a second dose (Table 1). Again there was a significantly higher geometric mean antibody concentration in the children who received a second dose of MMR (144.5 IU/ml) compared those who did not (37.1 IU/ml) ($P = 0.00001$). Of those who had received only a single dose, there was no significant difference in the proportion who were antibody negative or equivocal between children vaccinated at 12 months of age and children vaccinated at 13–17 months of age (40/180 versus 42/151, $P = 0.22$).

Table 1

The individual level antibody status of 389 children who received a single dose of MMR vaccine^{a,b}

	Measles/mumps/rubella antibody status							
	+/+/+	+/+/-	+/-/+	+/-/-	-/+/+	-/+/-	-/-/+	-/-/-
Observed	230	8	73	2	52	3	16	5
Expected (independent)	224.8	10.9	73.7	3.6	54.6	2.6	17.9	0.9
Expected (final model)	230.0	8.4	71.9	2.6	52.6	1.9	16.5	5.0

^a Positive (+), negative/equivocal (-).

^b Number observed; number expected if seropositivity for each component was independent, and number expected allowing for a proportion of complete vaccination failures.

IgM antibody to mumps was detected only 0.4% of the children who received a MMR second dose and in none of those who did not.

3.1.3. Rubella

The distribution of IgG antibody in the two groups is shown in Fig. 1c. Only a small proportion (1.0 and 3.6%) of those receiving a single dose of MMR were rubella IgG antibody negative or equivocal compared with none of those given a second dose. However, the geometric mean antibody concentration was significantly higher in the children who received a second dose of MMR (72.4 IU/ml, 95% CI 70.8–74.1) compared to those who did not (40.7 IU/ml, 95% CI 38.0–43.6) ($P = 0.00001$). Of those who had received only a single dose, there was no significant difference in the proportion who were antibody negative or equivocal between children vaccinated at 12 months of age and children vaccinated at 13–17 months of age (7/186 versus 9/151, $P = 0.49$).

IgM antibody to rubella was detected in 4.1% of the children who received an MMR second dose and in 2.8% of those who did not. This difference was non-significant ($\chi^2 = 0.69$, $P = 0.41$).

3.1.4. Individual level analysis of MMR seropositivity

The antibody status of the 389 children who received a single dose of MMR, is shown in Fig. 1; 41% had negative or equivocal levels to one or more antigens. After a second dose of MMR vaccine, this proportion dropped to <4% (Fig. 2).

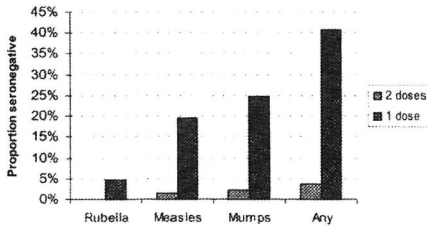


Fig. 2. Proportion antibody negative/equivocal to one or all of the three antigens by number of doses received. Comparing one dose group: 4 years post-dose 1 and two dose group: 6 weeks post-dose 2.

At the individual level, there was evidence of some dependence in the probability of seropositivity for each antigen ($\Delta Dev = 11.4$ on 4 d.f., $P = 0.02$) (Table 1). In particular, five children were negative or equivocal to all three antigens, higher than the number expected (0.9) if seropositivity to each vaccine component was entirely independent. However, after accounting for a small proportion (1%) of children who were complete vaccination failures, there was no evidence of any further dependence in the probability of seropositivity to each vaccine component ($\Delta Dev = 0.73$ on 3 d.f., $P = 0.87$) (Table 1).

3.2. National serological surveillance

For the birth-cohort born in 1989, the measles and rubella IgG antibody distributions, in 1994 before the November

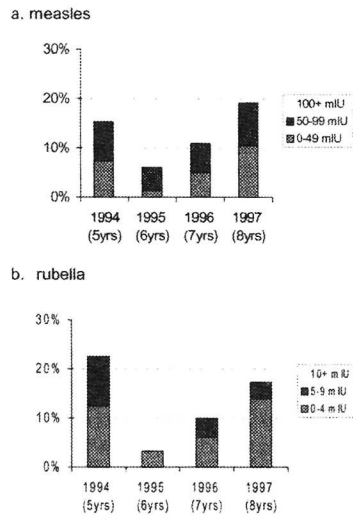


Fig. 3. (a) Proportion of age-cohort with low measles IgG antibody levels in cohort pre- and post-MR dose; (b) proportion of age-cohort with low rubella IgG antibody levels in cohort pre- and post-MR dose.

1994 MR campaign, and in the subsequent 3 years are shown in Fig. 3. A large decline in the proportion of the cohort with low or no detectable IgG antibody for measles and rubella is seen from before to after the MR campaign. There is a subsequent increase in the proportion with low or no detectable antibody over the two following years (1996–1997) back to the pre-second dose MR level. A similar observation is seen for the 1988 cohort (data not shown).

4. Discussion

This paper shows that 2–4 years after receiving a first dose of MMR vaccine, a significant proportion of pre-school children had measles and mumps IgG antibody levels below the putative level of protection, whereas few remained unprotected against rubella infection. These observations are consistent with previous immunogenicity studies in the UK [10] and Canada [25]. The higher protection afforded by a single dose of rubella vaccine is compatible with these studies where less than 1% of the study population were seronegative for rubella antibody and confirms the excellent immunogenicity of the RA 27/3 rubella strain [25].

We examined several factors that might influence seropositivity including age at vaccination and correlation with presence of antibody to the other MMR antigens. We found that pre-school children who had been immunised at 12 months of age were significantly less likely to have measles antibody than those vaccinated at 13–17 months. This is consistent with the vaccine efficacy observed in a measles outbreak in Canada [26]. However, we found no effect of age at immunisation on mumps or rubella seropositivity in pre-school children. We found no evidence for correlation of seropositivity to each antigen, other than that produced by a small excess of children negative or equivocal to all three antigens (1%). This contrasts with observations by Sauver *et al.* who found small but significant correlations in low antibody levels, with 0.3% of children seronegative to all three antigens [27]. The mechanism for our observations is unclear. The excess of children with low antibody levels to all three antigens may be caused by a small proportion of complete vaccination failures, due to impotent vaccine, incorrect delivery or individual factors. The independence of seropositivity in the remainder supports the suggestion that low antibody levels may be due to individual immunogenetic factors [28] or purely chance. The large proportion of children with low antibody levels for at least one antigen (41%) after receiving a single dose of vaccine supports the policy of administering a second dose of trivalent MMR vaccine to all children.

The vast majority of children who receive a single dose of MMR seem to be immunologically primed as evidenced by the lack of a significant IgM response to any of the three antigens following a second dose of MMR in the pre-school study. This significant increase in IgG levels but lack of an IgM response suggests that a large proportion of the observed population seronegativity after a single dose was due

to waning antibody levels (rather than primary vaccine failure). The 4–6-week time window from administration of MMR vaccine to serum sampling should have been adequate to detect the IgM increase associated with a primary immune response, as IgM antibody levels would not have declined significantly and a large proportion would still be expected to remain seropositive [29]. Our results conflict with published evidence from some other studies: Erdman *et al.* [11] found that amongst pre-school children who had received a single dose of vaccine, but had no detectable pre-existing antibodies, 33/36 developed IgM antibody following boosting with a dose of MMR. This suggests that the IgM assay used by Erdman was either more sensitive than ours, detecting the low levels of IgM that may be associated with secondary responses, or was less specific [30].

Administration of a second dose of MMR boosted IgG antibody to levels similar to those found 6 months after the first dose for measles and rubella and to even higher levels for mumps. This has been observed previously for each of measles [31,32], mumps [31,33] and rubella [31]. However, the increase in antibody levels does not seem to be sustained for either measles or rubella. The decline in measles and rubella antibody levels observed in our serological surveillance of children pre- and 2 years post-MMR second dose has also been documented in a longitudinal cohort study in Finland [34] and in re-vaccinated adolescents and young adults elsewhere [35,36].

How do these immunogenicity results correlate with clinical protection? Some authors have suggested that particular antibody concentrations are protective against measles disease [7]. Although it is clear from recent studies of measles outbreaks that vaccine efficacy is higher after two doses of MMR than after a single dose [37–39], within 3 years of receiving a second dose of measles containing vaccine, population antibody titres have returned to pre-second dose levels. These observations suggest that total IgG is not an entirely appropriate correlate of protection in highly vaccinated populations, since low antibody levels in vaccinated individuals are not indicative of lack of clinical protection.

With the introduction of two-dose MMR programmes in many countries with improved measles control, continued monitoring of long-term protection will be required particularly in the absence of boosting from wild virus circulating in the population. Measurement of IgG avidity, has been used to distinguish primary and secondary responses after vaccination and exposure to measles, mumps and rubella [40–42]. The role of antibody avidity testing in the evaluation of MMR vaccination strategies in a variety of populations merits further investigation.

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