

Humoral and cellular immune response after measles vaccination in Taiwan

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Measles immunoglobulin G (IgG) seroepidemiologic studies have been widely used to monitor the effectiveness of measles immunization programs in Taiwan. However, studies about cellular immunity against the measles virus have been lacking. This study surveyed cellular immunity after measles, mumps and rubella combined vaccine (MMR) immunization in Taiwan. Seventy six people between 1 and 80 years of age were enrolled. All patients lived in northern Taiwan, and none of them had immunodeficient disease. Every enrolled patient donated a tube of heparinized blood between January 2004 and June 2004 for cross-sectional studies of IgG seroepidemiologic and MMR-specific lymphoproliferative response. The results showed that the current 3-dose (measles \times 1 + MMR \times 2) measles immunization program induced slightly higher IgG seroprevalence (100% vs 85%, $p=0.244$) and a higher frequency of significant (stimulation indices ≥ 3) MMR-specific lymphoproliferative response (50% vs 15%, $p=0.044$) than a 2-dose (measles \times 1 + MMR \times 1) immunization program, although there was no difference in IgG titers and stimulation indices. Furthermore, the population aged older than 36 years (pre-immunization era) had higher IgG titers and seroprevalence, and similar MMR-specific lymphoproliferative responses to that of the population aged younger than 36 years (post-immunization era). In summary, with the limited data, the current 3-dose (measles \times 1 + MMR \times 2) measles immunization policy probably more effectively induces humoral and cellular immunity than the 2-dose (measles \times 1 + MMR \times 1) policy. Measles IgG seroprevalence in populations of different age groups exceeds nearly 90%. Measles has been eliminated temporarily in Taiwan. For a better understanding of the durability of vaccine-induced immunity and in order to establish the most appropriate immunization schedule, long-term and large-scale prospective studies of measles-specific seroepidemiology and cellular immunity will be needed.

Key words: Cellular immunity, immunoglobulin G, measles, MMR vaccine, seroepidemiologic studies

Although a voluntary, self-paid measles vaccine program has been available since 1968, measles had been endemic with a 2-year epidemic cycle in Taiwan until the introduction of the universal measles immunization program (2 doses, 1 at 9 months of age and the other at 15 months of age) in 1978. The most recent nationwide outbreak, occurring in 1988-89 [1,2], prompted health authorities to establish a national goal for measles elimination by 2000. Since then, a universal MMR (measles, mumps and rubella combined vaccine) immunization plan targeting primary and secondary schoolchildren (7-15 years old) was conducted in 1991-94, with coverage above 90%. Meanwhile, the 2-dose policy was adjusted to 1 dose of measles vaccine at 9 months of age and 1 dose of MMR at 15 months of age since 1991. Further immunization requirements have been implemented by screening vaccination records and

immunizing unvaccinated individuals among newcomers to primary school since 1991. In order to strengthen the effectiveness of measles immunization, the 2-dose policy was changed to a 3-dose policy (with the third dose of MMR at 7 years of age) starting in 1995.

Under this improved measles immunization strategy, the vaccine coverage with at least 1 dose of MMR vaccine in primary schoolchildren was greater than 95% in 1995. According to a community-based seroepidemiologic study of the Taiwan population in 1995-97, measles immunoglobulin G (IgG) seroprevalence (cut-off titer at 50 mIU/mL) reached >95% in schoolchildren (7-18 years old) and >98% in young adults (19-25 years old) but varied from 87% (in the mountain population) to 95% (in the urban population) in children aged 4-6 years old [3]. Although 2 small-scale outbreaks with 42 confirmed cases in 1994 and 27 cases in 2002 occurred in Taiwan, only limited confirmed cases were identified in 1992 (7 cases), 1993 (2 cases), 1997 (7 cases), 1998 (9 cases), 2000 (5 cases), 2001 (9 cases), and 2003 (6 cases) [4].

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In order to survey the immune status of measles in the Taiwan population and to monitor the effectiveness of the measles immunization program, IgG seroepidemiologic and MMR-specific lymphoproliferative response studies were conducted.

Materials and Methods

Seventy six people (including 40 admitted patients at National Taiwan University Hospital, 15 patients from outpatient clinics of National Taiwan University Hospital and 21 healthy volunteers) between 1 and 80 years of age were enrolled in this study. All patients lived in northern Taiwan, and all were without immunodeficient disease. Every enrolled patient donated a tube of heparinized blood between January 2004 and June 2004 for cross-sectional studies of IgG seroepidemiologic and MMR-specific lymphoproliferative response.

All samples were kept at room temperature and processed within 12 h of collection. Blood was centrifuged (1500 rpm for 15 min), and the plasma was removed and frozen at -20°C until used in assays. Blood cells were resuspended in the original volume of Hanks buffered salt solution (HBSS). Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Paque (Amersham Biosciences, Sweden) and were used to perform lymphoproliferative assay immediately.

A total of 78 sera were tested for measles IgG titers using a commercial enzyme immunoassay (EIA) kit (Dade Behring, Marburg, Germany). Each pair of antigen/control antigen wells was loaded with a test serum dilution of 1:231. Anti-measles virus reference serum (containing human IgG specific to measles virus) was run as the first and last sample in the assay series. The plates were read at 450 nm to obtain optical density (OD). For each test sample and reference sample, the difference in absorbance (DOD) was calculated as $\text{OD}_{\text{antigen}} - \text{OD}_{\text{control antigen}}$ and was converted to milli-international units per milliliter (mIU/mL) based on the α -method. It was valid only for the conditions where $\Delta\text{OD}_{\text{reference}}$ was within the range defined by the lower and upper margins given with each test set. The cut-off titer was set at 150 mIU/mL.

PBMC were washed once in HBSS and resuspended at $2 \times 10^6/\text{mL}$ in RPMI 1640 media (JRH Biosciences Lenexa, KS, USA) containing 50 $\mu\text{g}/\text{mL}$ gentamicin, 10 mM HEPES and 5% decomplexed human AB serum. Resuspended PBMC were distributed at 100 $\mu\text{L}/\text{well}$ on 96-well culture plates. The MMR vaccine (Priorix; GlaxoSmithKline, Belgium) containing the

attenuated Schwarz measles virus (titer $>10^{3.0}$ 50% tissue culture infecting dose [TCID_{50}]/mL), RIT 4385 mumps virus (titer $>10^{3.7}$ $\text{TCID}_{50}/\text{mL}$), and Wistar RA 27/3 rubella virus (titer $>10^{3.0}$ $\text{TCID}_{50}/\text{mL}$) was used as an antigen at the dilution of 1:10 and was distributed at 100 $\mu\text{L}/\text{well}$. Following 6 days of cultivation at 37°C in 5% CO_2 , 1.0 μCi of ^3H -thymidine was added to each well for an additional 24 h of culture. Then the cells were collected onto fiberglass paper using a cell harvester. ^3H -thymidine incorporation was determined on triplicate samples. Results were expressed as stimulation indices (stimulation index [SI] = antigen-induced ^3H -thymidine uptake/control ^3H -thymidine uptake). Stimulation indices ≥ 3 were considered to be significant.

Data were analyzed with the SAS statistical package (Version 8.2, SAS Institute, Cary, NC, USA). Measles IgG seroprevalence/MMR-specific lymphoproliferative response and their 95% confidence intervals (CIs) were obtained by assuming a binomial distribution. Since the distribution of measles IgG titer was positively skewed, we took the log transformation of it and assumed log measles IgG titer followed a normal distribution in each group (grouping either by age or by vaccination status). The statistical significance between the geometric mean titre (GMT) and stimulation indices of lymphoproliferative response was tested using analysis of variance (ANOVA). A 2-sample test for binomial proportions and the pairwise Bonferroni method were used for comparisons between groups.

Results

Measles IgG seroprevalence and GMT

Measles IgG seroprevalence and GMT increased with advancing age, except in the group aged from 16 to 25 years with low seroprevalence (75%) and low GMT (Fig. 1). If we divided these 76 cases into 2 groups [<36 years (i.e., vaccines) versus ≥ 36 years (i.e., without vaccination)], the seroprevalence was 87.5% and 100%, respectively ($p=0.026$) [Table 1]. The statistical significance between the GMT data was tested using ANOVA with p value <0.001 ($F_0 = 6.59$). The result suggests that the GMT between each age group is statistically different. When we classified the individuals into 2 age groups (<36 versus ≥ 36 years), their GMT values were also statistically different ($p<0.001$, $F_0 = 27.58$) [Table 2].

In order to evaluate the effectiveness of immunization, all cases were regrouped according to immunization status. Table 3 and Fig. 2 illustrate the vaccination

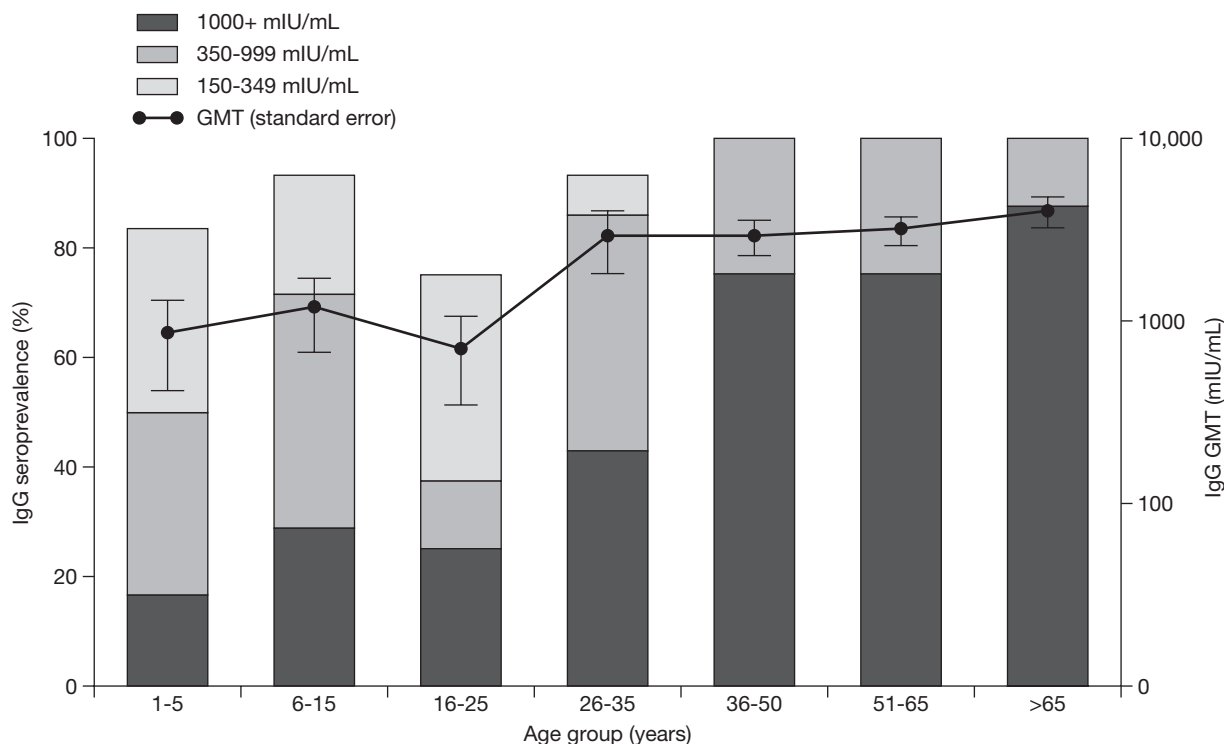


Fig. 1. Age-specific measles immunoglobulin G (IgG) seroprevalence and geometric mean titre (GMT).

status-specific measles IgG seroprevalence and GMT. The seroprevalence in the 3-dose group (measles \times 1 + MMR \times 2) was 100%, which was not significantly different from the rate in the 2-dose group (85%, $p=0.244$) or the non-vaccination group (100%, $p=1$). Using the pairwise Bonferroni method, we found no difference in the measles IgG titer between the 3-dose

group (measles \times 1 + MMR \times 2) and the 2-dose group ($p=1$). If we assumed that children aged <13 years had not been exposed to wild measles virus (the most recent nationwide outbreak occurring in 1988-89), the current 3-dose policy (measles \times 1 + MMR \times 2) seemed to induce a higher seroprevalence than the 2-dose policy, although without statistical significance.

Table 1. Age-specific measles immunoglobulin G (IgG) seroprevalence, geometric mean titer (GMT), and lymphoproliferative response (LP) in Taiwan, 2004

Age group (years)	No. ^a	IgG (%) ^b [95% CI] ^c	GMT \pm SD (mIU/mL)	LP (%) ^d [95% CI] ^e	SI (mean \pm SD)
1-5	12	83.3 (52-98)	851 \pm 1533	8.33 (0.21-38.48)	1.74 \pm 0.82
6-15	14	92.9 (66-100)	1191 \pm 1928	42.86 (17.66-71.14)	2.74 \pm 1.63
16-25	8	75.0 (35-97)	700 \pm 1008	50.00 (15.70-84.30)	4.94 \pm 3.92
26-35	14	92.9 (66-100)	2886 \pm 4050	35.71 (12.76-64.86)	3.47 \pm 3.48
36-50	12	100 (74-100)	2887 \pm 2179	25.00 (5.49-57.19)	3.65 \pm 3.23
51-65	8	100 (63-100)	3105 \pm 1557	37.50 (8.52-75.51)	4.84 \pm 6.43
>65	8	100 (63-100)	3914 \pm 2278	25.00 (3.19-65.09)	2.31 \pm 1.82
<36 ^e	48	87.5 (75-95)	1518 \pm 2661	33.33 (20.40-48.41)	3.07 \pm 2.77
\geq 36 ^f	28	100 (88-100)	3243 \pm 2026	28.57 (13.22-48.67)	3.60 \pm 4.10

Abbreviations: CI = confidence interval; SD = standard deviation; SI = stimulation index

^aTotal number of sera.

^bMeasles IgG seroprevalence (\geq 150 mIU/mL).

^cThe 95% CIs were calculated by assuming binomial distribution.

^dPrevalence of significant lymphoproliferative response (stimulation indices \geq 3).

^ePost-immunization era vaccines (<36 years).

^fWithout vaccination (pre-immunization era) [\geq 36 years].

Table 2. Analysis of statistical significance according to vaccine exposure in terms of measles immunoglobulin G (IgG) seroprevalence, geometric mean titer (GMT), prevalence of significant lymphoproliferative response (LP), and mean stimulation index (SI), respectively

Comparative groups	p^a (IgG seroprevalence)	p^b (GMT)	p^a (prevalence of significant LP ^c)	p (mean SI)
Two doses (measles × 1 + MMR × 1) vs 3 doses (measles × 1 + MMR × 2)	0.244	1.000	0.044	1.000
Age ≥36 years vs age <36 years ^d	0.026	<0.001	0.667	0.498

Abbreviation: MMR = measles, mumps and rubella combined vaccine

^aBy 2-sample test for binomial proportions.^bBy pairwise Bonferroni method.^cSignificant lymphoproliferative response (stimulation indices ≥3).^dPost-immunization era vaccines (<36 years) versus those without vaccination (≥36 years).

MMR vaccine-induced lymphoproliferative response

Table 1 summarizes all laboratory findings. The lowest frequency of significant lymphoproliferative response was 8% in the youngest group (1-5 years). Stimulation indices ≥3 were observed in 16/48 (33%) of the post-vaccine era population (<36 years) and 8/28 (29%) in the pre-vaccine era population (≥36 years) [$p=0.667$]. The mean stimulation indices of these 2 groups (<36 vs ≥36 years) were not significantly different (3.07 vs 3.60, $p=0.498$). Table 3 and Fig. 3 illustrate laboratory results classified according to vaccination status. The frequency of stimulation indices ≥3 in the 3-dose group (measles × 1 + MMR × 2) was 50%, which was significantly different from 15% in the 2-dose group (measles × 1 + MMR × 1) [$p=0.044$] and was not significantly different from the 29% value in the 0-dose group ($p=0.257$). The stimulation indices of the 3-dose (measles × 1 + MMR × 2) and 2-dose groups were not significantly different ($p=1$) [Table 2]. If we assumed that children aged <13 years had not been exposed to

wild measles virus, the current 3-dose policy (measles × 1 + MMR × 2) probably induced a higher prevalence of significant lymphoproliferative response than the 2-dose policy.

Discussion

Seroepidemiology is traditionally used to monitor the effectiveness of immunization programs [5-7]. In Taiwan, Chiu et al, found that the measles IgG seroprevalence (cut-off titer of 240 mIU/mL) in an urban population (Taipei City) from 1993 to 1995 reached 92-95% in schoolchildren (6-15 years old) but decreased to 88% in young adults (21-30 years old) [5]. A later study using a commercial EIA kit (cut-off titer of 50 mIU/mL), conducted by Lee et al reported that IgG seroprevalence in a 1995-1997 population reached >95% in schoolchildren (7-18 years of age) and young adults (19-25 years of age), but varied from 87-97% in children aged 1-6 years [3]. The most recent measles seroepidemiologic surveillance study using an EIA kit

Table 3. Vaccination status-specific measles immunoglobulin G (IgG) seroprevalence, geometric mean titer (GMT), and lymphoproliferative response (LP) in Taiwan, 2004

Vaccination status (age range, years)	No. ^a	IgG (%) ^b [95% CI] ^c	GMT ± SD (mIU/mL)	LP (%) ^d [95% CI] ^c	SI (mean ± SD)
0 dose [≥36]	28	100.00 (88-100)	3243 ± 2026	28.57 (13-49)	3.60 ± 4.10
1 dose [1-35] (measles or MMR)	16	93.75 (70-100)	2670 ± 3831	25.00 (7-52)	2.78 ± 3.09
2 doses [2-7] (measles × 1 + MMR × 1)	13	84.62 (55-98)	959 ± 1494	15.38 (2-45)	1.80 ± 0.92
3 doses [13-26] (measles × 2 + MMR × 1)	11	72.73 (39-94)	661 ± 907	54.55 (23-83)	4.97 ± 3.52
3 doses [7-13] (measles × 1 + MMR × 2)	8	100.00 (63-100)	1304 ± 2506	50.00 (16-84)	3.11 ± 1.70

Abbreviations: CI = confidence interval; SD = standard deviation; SI = stimulation index; MMR = measles, mumps and rubella combined vaccine

^aTotal number of sera.^bMeasles IgG seroprevalence (≥150 mIU/mL).^cThe 95% CIs were calculated by assuming binomial distribution.^dPrevalence of significant lymphoproliferative response (stimulation indices ≥3).

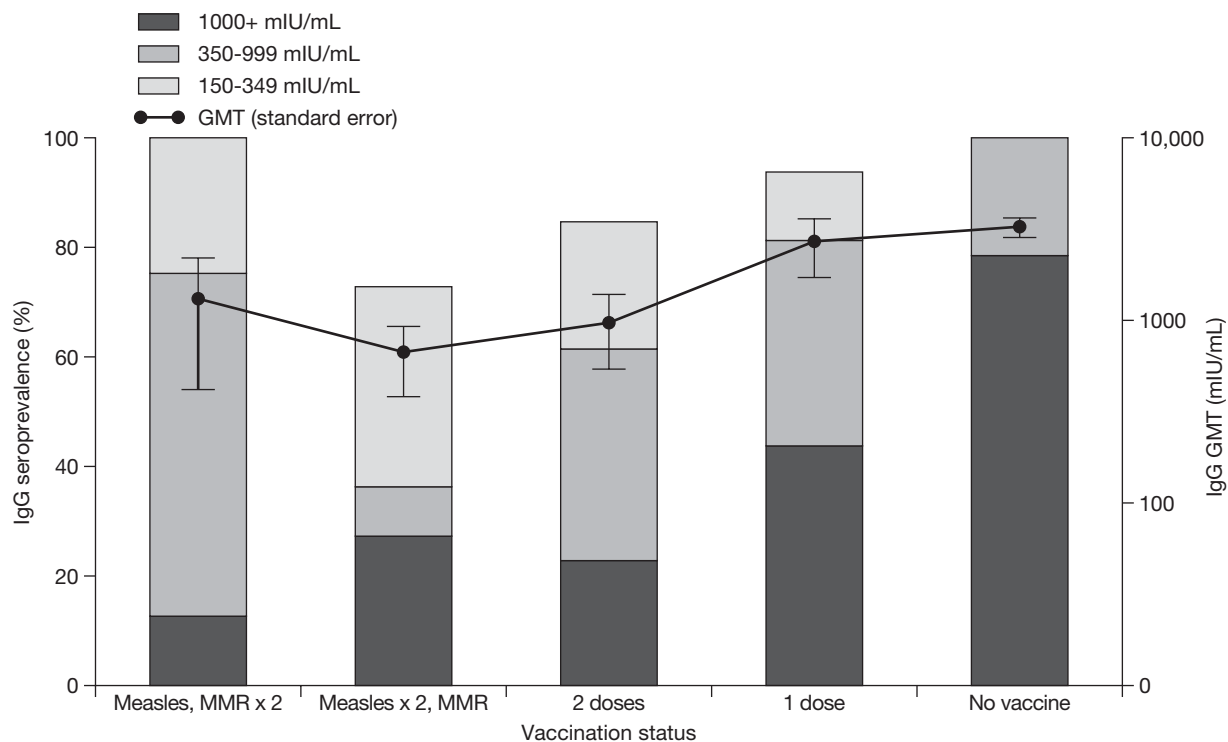


Fig. 2. Vaccination status-specific measles immunoglobulin G (IgG) seroprevalence and geometric mean titre (GMT). MMR = measles, mumps and rubella combined vaccine.

(cut-off titer of 100 mIU/mL), which was conducted by the Department of Health (Taiwan) based on 3609 cases aged 0-49 years in 1999, showed that the measles IgG titer was low (14.5-65.5%) in the 0- to 14-month-old population, increasing to >90% in other age populations, except for the 7- to 8-year-old group (88.2-89.2%). Our study reported that seroprevalence in most age groups reached >90%, which was similar to the previous study. Seroprevalence and IgG GMT in the pre-immunization era group (age ≥ 36 years) was higher than that of the post-immunization era group (age <36 years). This may be due to boosting effects from cyclic outbreaks of natural measles infection occurring before the universal immunization and vaccine failure in some cases. A previous study [2] confirmed the concept that a 2-dose vaccination program can induce significantly higher seroprevalence (98% vs 92%, $p < 0.01$) and a slightly longer half-life of measles IgG titer (61 vs 27 months, $p = 0.08$) than in the 1-dose vaccination program. Our study showed consistent results: the current 3-dose policy (measles $\times 1 + \text{MMR} \times 2$) induced a slightly higher seroprevalence than the 2-dose policy (100% vs 85%, $p = 0.244$).

Some may question the effect of the first dose measles immunization of younger infants because of the

immaturity of the immune system [8], as well as the potential for interference by passive antibodies [9,10]. Recent studies have confirmed that although there may be poor humoral immune response, early measles

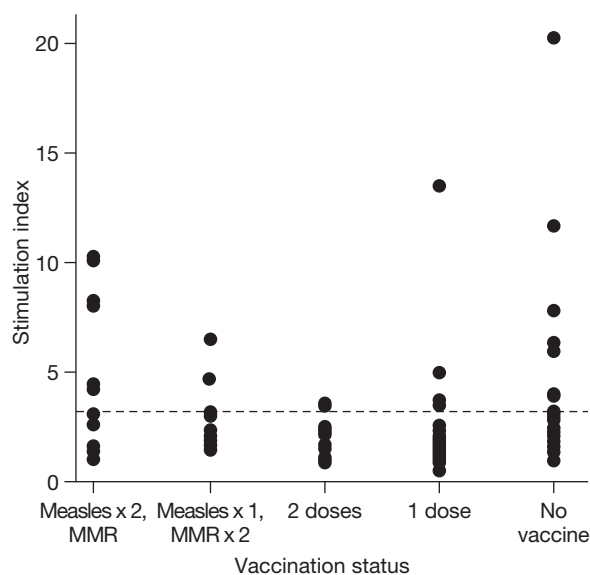


Fig. 3. Measles, mumps and rubella combined vaccine (MMR)-specific lymphoproliferative response according to vaccination status. Significant lymphoproliferative response: stimulation indices ≥ 3 .

immunization of infants at 6 months elicits T cell responses [11,12]. Measles protective immunity includes both humoral and cellular immune responses. Although the cut-off titer of 250 mIU/mL has been proposed to be a protective level of neutralizing antibody [13], some studies have shown that the vaccinees with low or undetectable antibody titers may not necessarily be susceptible to symptomatic measles infection [14-16]. Therefore, cellular immunity may contribute to protection against measles infection. We modified the lymphoproliferative assay method used by Bautista-López et al [17], and we used MMR vaccine as an antigen (to replace measles antigen), because the current 3-dose policy for measles immunization included MMR. Since the MMR vaccine is a mixture of measles, mumps and rubella virus antigens, and since seroepidemiologic surveillance in Taiwan in 1999 by the Department of Health reported high IgG seroprevalence in the population aged greater than 15 months (>90% for measles IgG, >88% for rubella IgG, and 46-85% for mumps IgG), the MMR-specific lymphoproliferative response can only represent the cellular immunity against any one or a combination of these 3 viruses, but not against measles virus alone. Lymphoproliferative responses in our study were detectable in 2/13 (15%) with a mean SI of 1.80 in children (2-7 years old with immunization at age of 9 months and MMR at age of 15 months), which was lower than 19/28 (66%) with a mean SI of 11.4 in children 5-8 years old receiving MMR at age of 1 year in the study conducted by Bautista-López et al [17]. This difference may be attributed to the difference in the stimulating antigen used in the assay. Our study showed the lowest lymphoproliferative response in 1- to 5-year-old children. No significant difference in the lymphoproliferative response existed between the <36-year-old and ≥36-year-old populations. We may assume children aged <13 years have not been exposed to wild measles, rubella and mumps viruses, because the most recent nationwide outbreaks of measles, mumps and rubella were in 1988-89, 1992 and 1992-93, respectively. Therefore, this study confirms that the current 3-dose policy (measles × 1 + MMR × 2) can induce a significantly higher prevalence of significant lymphoproliferative response than a 2-dose policy (50% vs 15%, $p=0.044$) and a slightly higher prevalence compared with the pre-immunization era population (50% vs 29%, $p=0.257$). We did not analyze the relationship between humoral and lymphoproliferative responses to measles virus, because the lymphoproliferative response in this study was

MMR-specific but not measles-specific. According to a previous study [17], surprisingly, there was no correlation between measles antibody titers and lymphoproliferative response.

A theoretical model of the transmission of measles implies that herd immunity thresholds in excess of 90% would protect society from large-scale measles outbreaks [18]. According to our study, under the current 3-dose measles immunization program, measles IgG seroprevalence reached >90% in most age groups, demonstrating better induced humoral and cellular immunity. Therefore, it appears that measles has been temporarily eliminated in Taiwan. Decay of vaccine-induced antibody titers under the condition of no boosting from wild measles virus, however, has been documented in cohort studies [19,20]. So reimmunization of those who failed to respond to the initial dose and those with low levels of antibodies is reasonable, and this practice does not cause any adverse consequences on immunologic function, except decreased leukocyte function [21]. For a better understanding of the durability of vaccine-induced immunity and the setting of an appropriate immunization schedule, long-term and large-scale prospective studies of measles-specific seroepidemiology and cellular immunity will be needed.

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