

Waning Antibody Levels and Avidity: Implications for MMR Vaccine-Induced Protection

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Background. The measles-mumps-rubella (MMR) vaccine is effective in eliciting a good antibody response. In addition to the amount of antibodies, the avidity of these antibodies might be important in protecting against disease.

Methods. The amount of circulating antibodies for measles, mumps, and rubella was measured with enzyme immunoassays, and the avidity of these antibodies was determined by urea dissociation. Three groups of twice-MMR-vaccinated individuals and 1 group of naturally infected individuals were studied. One vaccinated group (n = 71) was studied 6 months and 20 years after a second MMR vaccination.

Results. The antibody avidity indexes were high for measles and rubella but low for mumps. Twenty years after a second MMR vaccination, antibody levels for all 3 viruses waned. Also, the mean avidity index decreased by 8% for measles, 24% for mumps, and remained unchanged for rubella. Antibody avidity correlated with antibody concentration for measles. There was partial correlation for rubella and no correlation for mumps.

Conclusions. Measles and rubella induced high-avidity antibodies and mumps induced low-avidity antibodies after both vaccination and natural infection. Waning of both the concentration as well as the avidity of antibodies might contribute to measles and mumps infections in twice-MMR-vaccinated individuals.

The trivalent vaccine against measles, mumps, and rubella has been in use worldwide for decades and has reduced disease incidence very effectively. As a result of successful vaccination programs in many countries, there is an increasing number of individuals who rely solely on vaccine-induced immunity against measles, mumps, and rubella. Antibody avidity has been defined to be the overall strength of binding affinities between multivalent antigens and their antibodies or, in other words, their functional affinity [1]. Antibody avidity matures after immunization and natural

infection by the progressive increase in the amount of more specific, higher-affinity antibodies [2]. Maturation of measles, mumps, and rubella antibody avidities seems to be quite similar in duration (c. 6 months) after vaccination [3–5]. High-affinity matured antibodies are considered to be superior to low-affinity antibodies in biological reactions, including virus neutralization, and are associated with protection against disease. Lower-affinity antibodies have been associated with disease progression and reinfection [2].

Antibody avidity measurements have been used to distinguish acute infections from earlier infection as well as to distinguish primary vaccine failures from secondary vaccine failures in measles [3, 6–8], mumps [4, 9, 10], and rubella [11–14]. Low-avidity (LA) antibodies are detected in primary infections and primary vaccine failures, whereas high-avidity (HA) antibodies are considered a sign of earlier infection or vaccination and consequently also imply secondary vaccine failure and waning immunity.

In Finland a 2-dose measles, mumps, and rubella (MMR) vaccination schedule has remained unchanged

Received 14 March 2012; accepted 17 June 2012; electronically published 10 September 2012.

Presented in part: Avidity of Measles, Mumps and Rubella Antibodies Induced by MMR Vaccine. European Society for Pediatric Infectious Diseases, Brussels, Belgium, 10–13 September 2009. Poster 565.

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The Journal of Infectious Diseases 2012;206:1542–8

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DOI: 10.1093/infdis/jis568

from 1982 to 2010, with the first dose given at 14–18 months of age and the second dose at 6 years of age. The elimination of indigenous measles, mumps, and rubella was achieved by the mid-1990s [15, 16]. A cohort was recruited at the start of the MMR vaccinations in 1982 to study the persistence of vaccine-induced antibodies. Those in the cohort had few opportunities for natural boosters because the incidence of measles, mumps, and rubella declined rapidly after the start of the vaccination program [15]. The cohort has been studied extensively, and the 25-year-long antibody follow-up has shown that MMR vaccine-induced antibodies wane over time [17–20], as has been shown in other studies [21–23].

Vaccination can give rise to high levels of antibodies but these antibodies need to be functional against the pathogen. Also, factors such as antibody affinity, valence, and possibly species differences affect their neutralizing power [24].

The aim of this study was to investigate the change and correlation of antibody concentration and avidity over time after vaccination. Another objective was to determine if there is a difference between antibody avidity induced by MMR vaccination and natural infections. Another objective was to possibly obtain more information on avidity's role in protecting against MMR infection.

SUBJECTS AND METHODS

Study Subjects

The study was comprised of 4 groups. Following is a description of these groups.

There were 2 groups (1 and 2) from an MMR vaccination cohort studied since 1982. Group 1 ($n = 71$) consisted of individuals from a cohort recruited in 1982. All individuals in this group were seronegative for measles, mumps, and rubella before receiving 2 MMR vaccinations at 14–18 months and 6 years of age. Samples were taken 6 months (1987) and 20 years (2007) after the second MMR vaccination. The persistence of measles, mumps, and rubella antibodies in this group during the last 20 years has been described in detail in previous articles [17–20]. Group 2 ($n = 48$) included older individuals from the cohort recruited in 1982. This group had received a monocomponent measles vaccine (Rimevax containing the Schwarz strain) at c. 12 months of age, and all were seronegative for mumps and rubella before being vaccinated with MMR vaccine at 6 and 11–13 years of age. Samples were taken 18–20 years (2007) after the second MMR vaccination.

Group 3 ($n = 50$) included children aged 10–11 years born after the elimination of MMR diseases from Finland [15]. The samples were taken from residual sera collected 4–5 years after age for the second dose of MMR in 2005 at the Helsinki and Uusimaa hospital district laboratory. The vaccination status was not verified; however, vaccination coverage was >95%

during their lifetime and all individuals were positive for rubella immunoglobulin G (IgG), indicating vaccination.

Group 4 ($n = 50$), also collected from the same set of residual sera, were presumably naturally infected 50- to 59-year-olds not covered by the MMR vaccination program. The men in this group ($n = 24$) most likely received 1 dose of inactivated mumps vaccine (Enders strain) at the age of about 20 years as army recruits (compulsory army service).

MMR Vaccine Used

The MMR vaccine used for all vaccinations of groups 1, 2, and 3 was the MMR II (Merck) vaccine, which contains the Moraten strain of measles virus; the Jeryl Lynn strain of mumps virus; and the RA27/3 strain of rubella virus. Vaccination coverage in Finland for the first dose has remained high (>95%) since 1987 [25].

Ethical Approvals

The use of the residual sera as well as the cohort study was approved by the Hospital District of Helsinki and Uusimaa Ethical Committee of Epidemiology and Public Health in Helsinki, Finland. All cohort participants for the 25-year sample collection gave written consent.

Methods

Antibodies were tested with Enzygnost antimeasles/IgG, antimumps/IgG, and antirubella/IgG tests (Siemens, Germany) according to the manufacturer's instructions.

The Enzygnost antimeasles/IgG and antimumps/IgG assays with whole virus as antigen were modified for testing antimeasles virus and antimumps virus IgG avidity as follows. Serum samples diluted according to the manufacturer's instructions (1:231) were added on the plate in 4 wells: antigen and control antigen well for determining the amount of high-avidity antibodies and antigen and control antigen well for the avidity control for determining the total amount of IgG antibodies. The test was done according to manufacturer's instructions with the exception that after sample incubation, wells were emptied and 6 M urea was added to the avidity wells to remove any low-avidity antibodies. Wash solution was added to the avidity control wells. Plates were incubated for 3 minutes at room temperature and then washed 3 times. Thereafter the test was continued according to the manufacturer's instructions. The avidity index was calculated as a percentage [(urea-treated OD (optic density)/ untreated OD) \times 100]. Avidity for a sample was calculated only if the OD of the untreated well was ≥ 0.100 . Lower dilutions (1:2 and 1:4 of the original) were used when necessary to obtain OD values of ≥ 0.100 to be able to measure avidity. Avidity index values <30% were considered to indicate low avidity, 30%–50% intermediate avidity, and >50% high avidity of the antibodies in a sample. The use of 6 M urea was based on previous publications [4, 26] as well as on testing with acute

infection and earlier infection samples and different urea concentrations to best distinguish between high and low avidity (data not shown).

For rubella IgG avidity, the Euroimmun antirubella-virus enzyme-linked immunosorbent assay (IgG) avidity test (Lübeck, Germany) with whole virus as antigen was used according to the manufacturer's instructions. Avidity for a sample was calculated only if the OD of the untreated well was ≥ 0.100 . Avidity index values $<40\%$ indicated low avidity, $40\%–60\%$ intermediate avidity, and $>60\%$ high avidity of the antibodies of a sample.

Statistical Methods

The *T* test was used to determine the difference in means for antibodies and avidity indexes.

Correlation of antibodies and avidity indexes were calculated using Microsoft Excel.

RESULTS

Measles

There were no measurable measles IgG antibodies for 15.5%, 10.4%, 4%, and 0% of groups 1, 2, 3, and 4, respectively. The avidity index of measles antibodies was high in most samples in all study groups (Figures 1 and 2). All members of group 1 who had measurable antibodies after 20 years had high-avidity antibodies 6 months after the second dose of MMR vaccine; 20 years later all group 1 members still had either high- or intermediate-avidity antibodies (Figure 1). In 20 years the geometric mean titer of antibodies decreased by 58% and the mean avidity index decreased by 8% (Figure 1, Table 1). Group 2 had the lowest mean level of antibodies, but all members were of high avidity. In group 3 (10- to 11-year-olds) the avidity index was significantly higher than in group 1 but comparable to group 2. The mean level of antibodies for group 3 was 42% higher than for group 1. The lowest proportion of high-avidity antibodies was found in the naturally infected group 4, which had the highest mean level of antibodies (Table 1).

Mumps

There were no measurable mumps IgG antibodies for 23%, 10%, 26%, and 8% of groups 1, 2, 3, and 4, respectively. The avidity index of mumps antibodies was low in most of the samples in all study groups (Figures 1 and 2). The majority of those in group 1 had low-avidity antibodies as soon as 6 months after the second MMR vaccination. The proportion of low-avidity index samples did not change a great deal in 20 years in group 1, even though the mean avidity index decreased by 24%. The geometric mean antibody titer decreased by 75% in 20 years (Figure 1, Table 1). Group 2 had a significantly higher mean avidity index than group 1 but a comparable mean level of antibodies (Table 1). The most recently

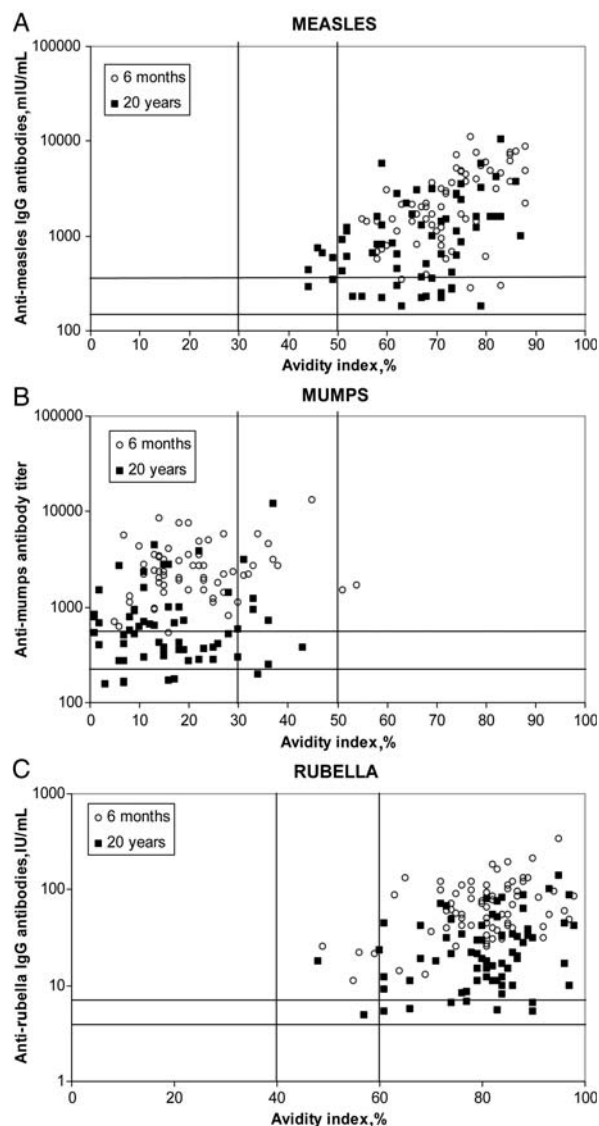


Figure 1. Antibody concentration vs antibody avidity index for (A) measles, (B) mumps, and (C) rubella for the samples taken from group 1 6 months and 20 years after the second MMR vaccination. The horizontal lines represent the cutoffs for negative and positive antibody concentration and the vertical lines represent the cutoffs for low and high antibody avidity index.

vaccinated group 3 had the highest proportion of intermediate- to high-avidity antibodies (32%), even though the antibody level was comparable to the 20-year level of group 1. Group 4 had the highest geometric mean antibody level (excluding the 6-month sample of group 1), but the proportion of individuals with low-avidity antibodies was also high in this group. The geometric mean antibody titer was 60% and the mean avidity index was 38% higher in men in group 4 than in women (Table 1).

Rubella

All individuals in all study groups had measurable antibodies against rubella, and the avidity index could be calculated for all.

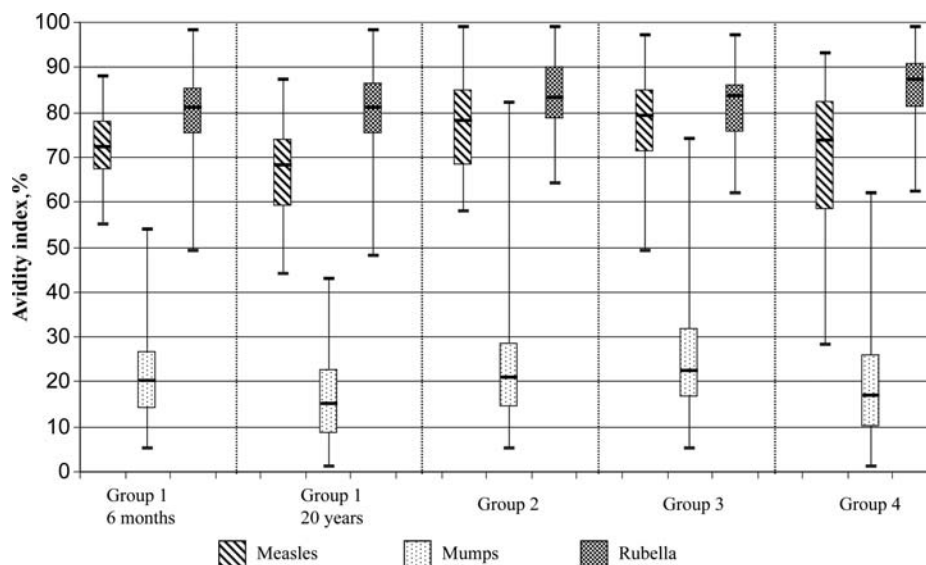


Figure 2. Avidity indexes for groups 1, 2, 3, and 4, including their minimum, lower quartile, median, upper quartile, and maximum.

The avidity index of rubella antibodies was intermediate to high in all samples from all study groups (Figures 1 and 2). The avidity index in group 1 remained unchanged in 20 years, even though the geometric mean titer of antibodies decreased

by 65% (Figure 1, Table 1). All individuals in group 2 had high-avidity antibodies, and the mean amount of antibodies was 40% larger than in group 1. The mean amount of antibodies as well as the mean avidity index in group 3 was

Table 1. Geometric Mean Antibody Concentrations and Mean Avidity Indexes for Groups 1, 2, 3, and 4 for Measles, Mumps, and Rubella and the Number and Percentage of Low-, Intermediate-, and High-Avidity Samples

	No.	Geometric Mean	Avidity Index			
		mIU/mL Geomean	Mean	Low (%)	Intermediate (%)	High (%)
Measles						
Group 1 6 mo	66	2029	72 ^a	0	0	66 (100)
Group 1 20 y	66	853	66	0	6 (9)	60 (91)
Group 2	45	736	77 ^a	0	0	45 (100)
Group 3	49	1209	78 ^a	0	1 (2)	48 (98)
Group 4	50	4303 ^a	70	1 (2)	7 (14)	42 (84)
Mumps						
	No.	Titer Geomean	Mean	Low (%)	Intermediate (%)	High (%)
Group 1 6 mo	62	2338	21 ^a	51 (82)	9 (15)	2 (3)
Group 1 20 y	62	595	16	52 (84)	10 (16)	0
Group 2	46	609	22 ^b	36 (78)	9 (20)	1 (2)
Group 3	44	685	26 ^a	30 (68)	11 (25)	3 (7)
Group 4	45	1391	20	35 (78)	9 (20)	1 (2)
Women	21	694	15	19 (90)	2 (10)	0
Men	24	1733	24 ^a	16 (67)	7 (29)	1 (4)
Rubella						
	No.	IU/mL Geomean	Mean	Low (%)	Intermediate (%)	High (%)
Group 1 6 mo	71	60	80	0	4 (6)	67 (94)
Group 1 20 y	71	21	80	0	3 (4)	68 (96)
Group 2	48	40 ^a	84 ^b	0	0	48 (100)
Group 3	50	38 ^b	82	0	0	50 (100)
Group 4	50	71 ^a	85 ^a	0	0	50 (100)

^a Significant difference with a 99% confidence level to group 1 at 20 years.

^b Significant difference with a 95% confidence level to group 1 at 20 years.

comparable to that of group 2. Group 4 had the highest mean avidity index as well as the highest mean level of antibodies (Table 1).

DISCUSSION

The 3 components of the MMR vaccine were found to give very different antibody avidity responses, namely, measles and rubella components gave rise to high-avidity and mumps to low-avidity antibodies, irrespective of the nature of the antigenic stimulus (Figure 2). There was waning in the levels of antibodies against all 3 viruses over time after vaccinations. The antibody concentration correlated with the antibody avidity in all groups for measles and in all groups except for group 2 for rubella. The antibody concentration did not correlate with antibody avidity in any groups for mumps.

Although given in the same vaccination, the 3 components of the MMR vaccine produce quite different antibody responses and so are discussed separately here.

Measles

A correlation was found between measles IgG concentration and avidity index in all 4 study groups. Another recent study also showed the slow decline of high-affinity antibodies but found no correlation to the antibody concentration. Because the size of the group studied was smaller in the other study ($n = 30$), the influence of individual variation could explain the opposing results [27].

An additional vaccination does not necessarily result in a larger amount of antibodies in the long run but might improve their quality. This is shown by group 2 having a significantly higher avidity index but lower IgG concentration for measles than group 1 20 years after the second MMR vaccine dose (Table 1).

Somewhat concerning are the results of the most recently vaccinated group 3. Those in the group have lived their lives in an environment that can be considered completely free of natural boosters. As soon as 5 years after the second dose of MMR vaccination, 4% of the individuals were seronegative and 14% low positive for measles. However, the measurable antibodies in this group were of high avidity except for that of 1 individual.

The high avidity of antibodies might compensate for the low antibody concentration to some extent and vice versa. It has been suggested that there might be an avidity threshold and that antibodies below this threshold would require very high *in vivo* concentrations for effectiveness [28]. One could assume that a high concentration of high-avidity antibodies would give protection against infection, but there have been cases of measles with a high level of antibodies and a high avidity index [6, 26, 29–32]. One reason for the insufficiency of high-concentration high-avidity antibodies to fight against infection could be their inability to neutralize all virus

genotypes [6, 29]. However, there seems to be a correlation between avidity and neutralization for at least some genotypes [33].

A likely factor in protecting against measles is time after vaccination; high-avidity vaccine-failure patients tended to have a longer interval after vaccination than low-avidity vaccine-failure patients [8, 31]. Even though the proportion of individuals in group 1 with both low-concentration and low-avidity index antibodies for measles 20 years after the second vaccine dose is quite low, there is reason for concern regarding the persistence of vaccine-induced protection against disease. Because vaccination gives rise to high-avidity antibodies in a vast majority of vaccinees, it can be assumed that natural infection would do the same. If this is true, there seems to be some waning of antibody avidity after natural infection; 16% of group 4 had a low or intermediate avidity index. Whether this is of concern regarding the protection against reinfection is unclear, especially with the far higher level of antibodies induced by natural infection(s) than by vaccination.

Mumps

Of the 3 components of the MMR vaccine, the mumps component is clearly the least effective in eliciting a response that would give rise to antibodies of high avidity, as shown by group 1 having mostly low-avidity antibodies as soon as 6 months after the second vaccination. Very few individuals in any of the groups studied had high-avidity antibodies for mumps. Mumps is also a poor inducer of lasting high-concentration antibodies; the 20-year follow-up shows the biggest decrease of the 3 vaccine components for both mean antibody titer (65%) and mean avidity index (24%) for mumps antibodies. Whether the time after vaccination is connected to the increased risk of disease is questionable [34]. There was no correlation between antibody concentration and avidity in any of the groups, which is in agreement with a previous study [35].

Because all individuals in groups 1 and 2 were seronegative for mumps before the first dose of MMR vaccine, there must have been some difference between the groups due to of the fact that those in group 2 were older. Group 2 had a geometric mean antibody titer that was similar to that of group 1, but their mean avidity index was significantly higher (Table 1). The higher avidity index of group 2 might indicate that they have had more boosters from the still circulating viruses at the end of the 1980s and early 1990s due to more contacts in school or by traveling or it might indicate that being older at the time of vaccination gives rise to better-quality antibodies.

Similar to measles, the results for mumps for group 3 give rise to concern. In a booster-free environment the mean antibody concentration of group 3 for mumps was already comparable to that for group 1 only 5 years after the second dose of MMR vaccine. The mean avidity index for group 3 was found

to be the highest of all groups for mumps, and the group had the highest proportion of intermediate- to high-avidity antibodies. However, mumps avidity seems to be a poor indicator of protection, and a previous study [10] found no difference in the avidity indexes between a group of students exposed to mumps that were unaffected and those who showed clinical symptoms. The findings of this study provide more evidence as to why there is an increase in the report of mumps in twice-MMR-vaccinated individuals [34, 36].

The results of this study show that even though the mean antibody titer for mumps was highest for group 4, naturally acquired antibodies are mostly of low avidity. The men in group 4 had both a higher level of antibodies and a higher avidity index for mumps than the women. The difference is most likely due to the fact that the men in this group received a monocomponent mumps vaccination in the 1960s or 1970s as army recruits. This indicates that contrary to measles, an additional mumps vaccination with a different mumps virus strain may boost both the amount as well as the avidity of the antibodies. Mumps reinfections [37, 38] can be expected considering the low concentration and avidity that exist after natural infection, as seen in the women of group 4.

Rubella

Rubella provides an excellent antibody response after both vaccination and natural infection.

In contrast to measles and mumps, the antibody avidity for rubella does not seem to wane since there was no change in the mean avidity index during the 20-year follow-up, even though there was a 65% reduction in the geometric mean antibody concentration and 24% of the individuals had low positive antibodies (≤ 10 IU/mL).

The antibody concentration and avidity were found to correlate for rubella, except for the samples taken from group 1 20 years after the second MMR vaccination, which could indicate a faster waning of antibody concentration for group 1 than for group 2.

Again the question arises, did group 2 have more opportunities for booster effects from wild viruses or was there some advantage of the higher age at vaccinations since the geometric mean antibody level as well as the mean avidity index of group 2 was significantly higher than that of group 1? The finding that the antibody level as well as the avidity index of group 3 was lower than that of group 2 could also support the concept of an advantage of vaccinating older individuals.

The mean avidity index for the naturally infected group 4 was the highest of all groups studied, with all individuals having high-avidity antibodies for rubella. There were no individuals with low-avidity antibodies in the vaccinated groups. This was not found in a study where high-avidity indexes for rubella were not observed after vaccination but only after primary infection [12]. The differences in the study methods

and the criteria defining low (<40% vs <70%) and high avidity (>60% vs >90%) are probably the main reasons for the differing results.

Very few possible rubella reinfections have been reported [39–41]. Also, the reports for twice-vaccinated rubella cases are rare [42], which gives assurance to the effectiveness of both the rubella component of the MMR vaccination and the antigenic stimulus of the wild virus.

Although there is an indication of a link between higher avidity and protection against disease [2], the amount that waning of antibody avidity increases the risk of secondary vaccine failure and in turn the risk of infection or the risk of reinfection is not clear. It seems that even though higher antibody avidity could, to some point, compensate for the smaller amount of antibodies and vice versa, the time after immunization is also important. The correlation of antibody avidity with virus neutralization needs to be studied further, especially with different wild-type strains.

At least in Finland, herd immunity due to a high-coverage 2-dose vaccination program has kept the country free of measles, mumps, and rubella outbreaks for 15 years (until the end of 2010). The rubella component of the MMR vaccine seems to protect well against disease with a 2-dose program, and it is likely that no additional measures are required. However, the situation with measles and mumps is changing. The number of people with only vaccine-induced immunity and waning antibody concentrations as well as waning avidity is increasing. This is why we are likely to see, perhaps in the near future, an increase in the number of twice-vaccinated individuals infected with measles and mumps due to secondary vaccine failure, even in countries with very high vaccination coverage.

Notes

Financial support. This study was funded by the National Institute for Health and Welfare, Finland.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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