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Comparative Analysis of Titers of Antibody against Measles Virus in Sera of Vaccinated and Naturally Infected Japanese Individuals of Different Age Groups

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The anti-measles virus (MV) antibody titers in the sera of vaccinees and naturally infected individuals of different age groups were measured to help assess the efficacy of the current MV vaccination in Japan. Neutralizing (NT) antibody titers induced by vaccination were $2^{3.2}$ times lower than those induced by natural infection and declined significantly by age 20. The once-decreased NT antibody titers of the vaccinees increased $2^{3.6}$ times during their twenties to titers comparable to those of naturally infected individuals of the same age, implying the possible occurrence of natural infection in vaccinees with decreased anti-MV immunity. Although the current field strains in Japan, types D3 and D5, were reported to differ antigenically from each other and from vaccine strains (type A) to some extent, as demonstrated by different reactivities to monoclonal antibodies, the sera of vaccinees neutralized the two types of field strains and the vaccine strain with the same efficiency. This result suggests that the current vaccine strain would be suitable to elicit protection against types D3 and D5, as long as viral antigenicity is concerned. However, when compared at given hemagglutination inhibition titers, NT antibody titers of vaccinees were $2^{1.1}$ to $2^{3.2}$ times lower than those of naturally infected individuals, suggesting a qualitative difference(s) of anti-MV antibodies between the two groups. It should be emphasized that protective immunity induced by the one-dose vaccination currently implemented in Japan may not be strong enough to ensure lifelong immunity. A two-dose vaccination program with higher vaccination coverage needs to be considered in order to effectively control measles in Japan.

Measles virus (MV), a member of the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, genus *Morbillivirus*, is the causative agent of measles, an acute systemic infection of, in most cases, young children. Measles is responsible for 10% of deaths from all causes among children less than 5 years old (18), causing an estimated 1,000,000 deaths each year worldwide. The most common cause of death is viral or superimposed bacterial pneumonia. Postinfectious encephalitis occurring in 0.1% of reported measles cases is also a serious problem, with death and residual neurologic damage in 15 and 25% of the cases, respectively. The virus can also cause, though not frequently, an incurable slow virus infection called subacute sclerosing panencephalitis (22, 29). Although the virus is highly contagious and easily transmitted, measles can be effectively prevented by attenuated live vaccine. Moreover, there is no known animal reservoir capable of sustaining transmission. Therefore, the World Health Organization has identified measles as one of the next likely candidates for eradication. Due to an intensive measles vaccination program and complete measles surveillance by the Pan American Health Organization, the incidence of measles is now very low in Latin America and the Caribbean, and the elimination of this disease from the Americas appears to be an achievable goal (4). In May 2000, it was concluded that the United States was no longer an area of measles endemicity (5). In Japan, on the contrary, the number of measles patients reported by 3,000 pediatric sentinel clinics under the Infectious Disease Control Law reached more than 22,000 (18 in 100,000) in the year 2000, with a predicted number for the near future being 5 to 10 times more (10). The most probable reason for the measles epidemic is insufficient vaccination. The measles vaccine has been given in Japan since 1978 by a routine immunization program, under which the vaccine is given in one dose scheduled at 12 to 90 months of age, with a recommendation for vaccination at 12 to 24 months. Vaccination coverage, however, has been only 75 to 81% in recent years (10). This is reminiscent of the situation in the United States in the 1980s, when measles outbreaks frequently occurred under a one-dose vaccination program with low (<70%) coverage (3). Moreover, vaccination coverage for one-year-old children in Japan is particularly low, with 48% of them being antibody-negative. It is thus estimated that ca. 1,000,000 children under 2 years old are susceptible to measles. As a result, about a quarter of reported measles cases occur at 12 to 24 months of age, followed by 10 to 15% at 6 to 11 months (10). It is also reported that nearly 100 children die of measles and/or its complications each year in Japan. To improve measles control in Japan, implementation of a better vaccination program is urgently required (6). For this purpose, precise information on the current anti-MV immune status in Japan is indispensable.

In addition to the low vaccination coverage, primary and secondary vaccine failures are another important issue to be considered. The protective immunity induced by vaccination

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TABLE 1. Anti-MV antibody titers of vaccinees and measles-infected individuals

Immunization status of subject	Age (yr)	Mean ± SD of antibody titer (Log_2)						
		NT			HI			
Vaccinated	≤9	4.8 ± 1.5	$P < 0.05$	$P < 0.0001$	3.7 ± 1.4	$P < 0.05$	$P < 0.0001$	
	10–19	3.3 ± 1.0	$P < 0.01$	$P < 0.05$	2.0 ± 0.0	$P = 0.09$	$P < 0.01$	
	20–29	6.9 ± 1.7			4.3 ± 2.3			
Naturally infected	≤9	8.0 ± 1.1	$P < 0.0001$	$P = 0.47$	5.4 ± 1.3	$P < 0.01$	$P = 0.61$	
	10–19	5.0 ± 1.0	$P < 0.05$		3.4 ± 0.5	$P = 0.17$		
	20–29	7.6 ± 2.2			5.0 ± 2.4			

may not be lifelong without being boosted by an exposure, mostly subclinically, to a naturally circulating virus. Due partly, if not entirely, to the secondary vaccine failure, the numbers of measles cases among adolescents (10 to 19 years old) and adults (20 to 39 years old) in Japan increased 1.7 times and 2.3 times, respectively, in 2001 over the previous year (10). Also, the latest studies have demonstrated the heterogeneous nature of MV, such as variations in hemagglutination (HA) activities (23, 28) and electrophoretic mobility (21) of the H protein, variations in reactivity to monoclonal antibody (13), and nucleotide sequence diversity (11, 25, 26, 34) between vaccine strains and currently circulating field strains. It is necessary, therefore, to compare the viral immunogenicity to humans between the two types of viruses and evaluate the protective efficacy of vaccine strains against currently circulating MV strains. In the present study, we analyzed antibody responses, induced either by vaccination or by natural infection, to the vaccine strains and currently circulating field strains of MV. We report here that, although anti-MV antibodies induced by vaccination neutralized the vaccine strain and field isolates with practically the same efficiency, neutralizing (NT) titers of vaccine-induced antibodies were lower than those induced by natural infection and declined significantly over decades. We also report that vaccine-induced antibodies with given hemagglutination inhibition (HI) titers had lower NT titers than did antibodies induced by natural infection.

MATERIALS AND METHODS

Participants. Serum samples were randomly collected from residents aged 3 months to 82 years who visited designated clinics around Osaka Prefecture, Japan, for unrelated reasons between May and October 2000. The subjects or their parents were questioned as to whether the subjects had contracted measles and whether they had received vaccination in the past. The vaccination histories were determined based principally on certificates in officially issued mother-and-baby notebooks and/or clear memories. When needed, physician records were also referred to. Subjects with uncertain statements and gamma globulin recipients were excluded. Of the 189 total participants (85 males and 104 females), 85 had received and 104 had not received MV vaccination. Seventy-seven had contracted measles and 112 had not. All the subjects belonged to middle-class families, with the usual Japanese standard of living and under normal nutritional conditions. Informed consents were obtained upon collection of serum samples.

Serological tests. NT antibody assay was performed according to Ward et al. (32) with minor modifications. Briefly, 25 μl of twofold serial dilutions of test sera was mixed with 25 μl of MV (Mvi/Osaka.JPN/29.99 [type D3], Mvi/Osaka.JPN/40.99/1 [type D5], or Edmonston [type A]) containing 75 cell-infecting units (comparable to PFU) in 96-well microtiter plates and incubated at 37°C for 1 h. The mixture was then mixed with 50 μl of suspension of B95a, a marmoset B-lymphoblastoid cell line (13), and incubated in 5% CO_2 at 37°C overnight. After being fixed with 100% ethanol, the cells were immunologically stained with blended mouse monoclonal antibodies against MV H and M proteins (Chemicon International Inc., Temecula, Calif.) and peroxidase-conjugated goat anti-mouse immunoglobulin G followed by chromogenic reaction using 3,3′-diaminobenzidine tetrahydrochloride. NT antibody titers were expressed as the highest serum dilutions with which MV infection was completely inhibited.

HI antibody titers were measured by a standard microtiter method using African green monkey red blood cells and the Toyoshima strain (type A) of MV.

Genotypes of the MV strains used were verified by sequence analysis at the end of this study.

Statistical analysis. NT and HI antibody titers were log transformed and were statistically analyzed. Student's and Welch's *t* tests and the Mann-Whitney U test were used to compare differences in antibody titers between two groups. Correlations were evaluated using the Spearman's correlation coefficient by rank test. Statistical significance was defined as *P* values of <0.05.

RESULTS

Anti-MV NT antibodies induced by vaccination or natural infection. Subjects younger than 9 years old who had received MV vaccination without a past history of measles possessed significantly lower levels of NT antibodies against MV than did those who got the antibody through natural infection at the same age (Table 1). It should also be noted that the mean antibody titer of one-year-old vaccinees was significantly lower than that of vaccinees 2 to 9 years old (3.9 ± 1.0 [mean ± standard deviation] and 5.1 ± 1.5, respectively; $P < 0.01$). The antibody titers of the vaccinees gradually declined as the children grew, until subjects reached the age of 15 to 19 years (Fig. 1). However, the titers of the vaccinees increased again during their twenties to levels comparable to those of the subjects who had contracted measles in the past. In general, NT antibody titers of subjects who had contracted measles were comparably high, with a minimum titer being 4.0 log_2 NT units/ml. Although the antibody titers decreased significantly at age 10 to 19 years, they still maintained a level high enough for protection, which was equivalent to what was induced by vaccination (5.0 ± 1.0 and 4.8 ± 1.5, respectively; $P = 0.74$) (Table 1). As was the case with the vaccinees, a significant increase in the antibody titers during their twenties was observed for naturally infected subjects.

Among the vaccinated subjects, only one (Fig. 1; see also Fig. 3) was confirmed to be a primary vaccine failure, as he had neither NT nor HI antibodies detectable after vaccination. For seven subjects who contracted measles after having received the vaccination (Fig. 1; see also Fig. 3), it was not determined

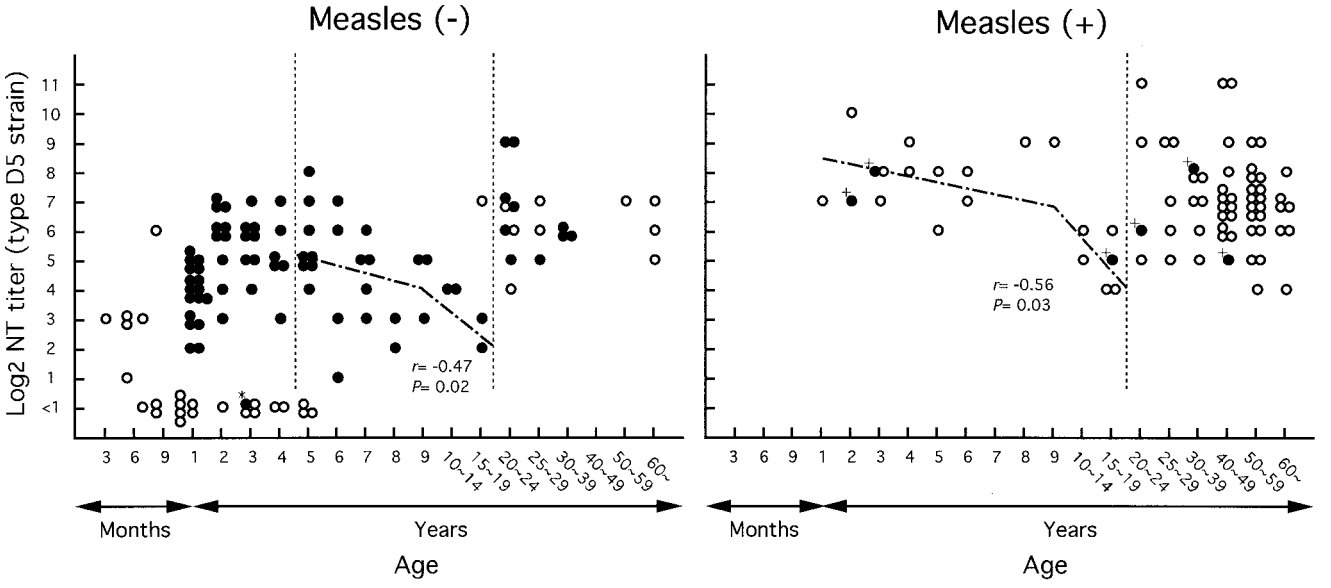

FIG. 1. NT antibody titers against MV in different age groups. NT titers were determined as described in Materials and Methods by using a field strain (type D5). Left and right panels show subjects without and with measles history, respectively. Correlations between NT titers and age (5 to 19 years for vaccinees and 1 to 19 years for MV-infected subjects) were evaluated by Spearman's correlation coefficient. ○, nonvaccinees; ●, vaccinees; ∗, primary vaccine failure; +, primary or secondary vaccine failure.

whether they did not show seroconversion at all (primary vaccine failure) or if they lost the protective levels of antibody once obtained by vaccination (secondary vaccine failure). Maternal NT antibody declined gradually and became undetectable at 8 months after birth in all but one of the subjects tested.

Reactivity of vaccine-induced and natural infection-induced anti-MV antibodies to various genotypes of MV. It has been reported that MV can be divided into a number of genotypes (19, 20, 33), some of which may exhibit different antigenicity (13, 27). Therefore, we measured antibody titers against different genotypes of MV, such as A, D3, and D5. The genotype A includes the Edmonston strain, a standard strain of MV, which is the parental strain of the current vaccine strain. The genotypes D3 and D5 represent the majority of field strains that are currently circulating in Japan (11, 25, 26, 34). NT antibody titers against the genotype D5 were practically the same as those against the genotype D3 (data not shown) and were in good correlation with those against the genotype A, regardless of whether subjects had been vaccinated or naturally infected (Fig. 2).

Difference in the NT-HI relationship of anti-MV antibodies in vaccinated and naturally infected individuals. The HI test was less sensitive than the NT test in detecting low levels of anti-MV antibodies. Consequently, a substantial number of the subjects, i.e., 35% of the vaccinees and 22% of those who had contracted MV by natural infection, were regarded as negative for anti-MV antibodies by the HI test (Fig. 3). The distribution pattern of HI titers in different age groups was basically the same as that for the residents in the eastern part of Japan (30). We noticed that some of the naturally infected individuals with high NT titers, but not vaccinees with high NT titers, tested negative for HI antibodies. Therefore, we analyzed the relationship between NT and HI titers and compared the results for the vaccinees and the naturally infected individuals. Since vaccinees more than 20 years old, who had significantly higher titers of anti-MV antibodies than vaccinees 10 to 19 years old, had possibly been infected by field strains of MV due to the decline in the antibody titers during their teens (secondary vaccine failure), they were excluded in this analysis. As for

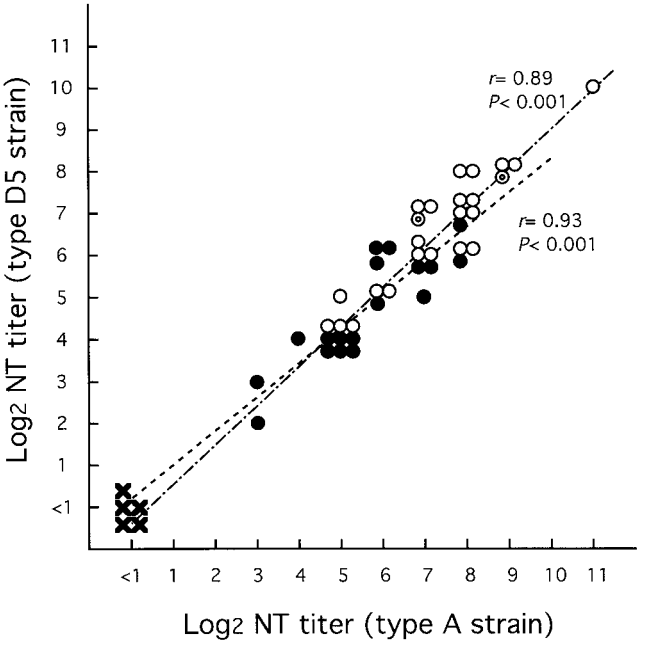

FIG. 2. NT antibody responses to different MV strains. NT titers against a field strain (type D5) were compared with those against the Edmonston strain (type A). Correlations between NT titers against type A and type D5 strains were evaluated by Spearman's correlation coefficient test for subjects without (- -) and with (– -) measles history. ✕, nonvaccinees without measles history; ●, vaccinees without measles history; ○, nonvaccinees with measles history; ◎, vaccinees with measles history.

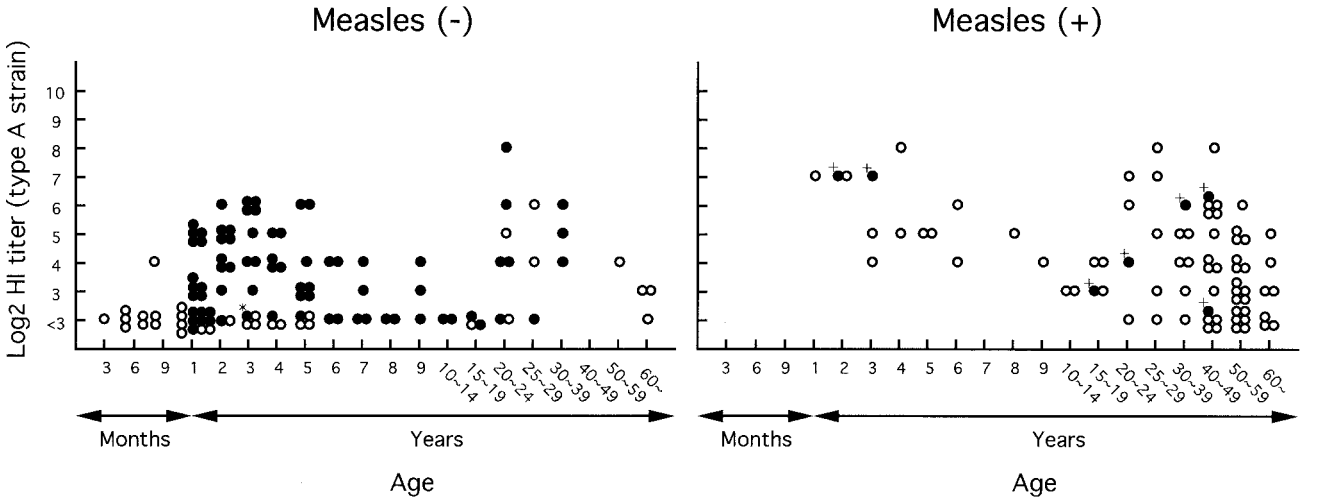

FIG. 3. HI antibody titers against MV in different age groups. HI titers were determined by the microtiter method using the Toyoshima strain (type A). Left and right panels show subjects without and with measles history, respectively. ○, nonvaccinees; ●, vaccinees; ∗, primary vaccine failure; +, primary or secondary vaccine failure.

naturally infected subjects, all the age groups were included. When compared at given HI titers, NT antibody titers of vaccinees were significantly lower than those of naturally infected individuals (Fig. 4). Practically the same result was obtained when age-matched vaccinated children and naturally infected children were compared (data not shown).

DISCUSSION

NT antibodies of vaccinees in different age groups demonstrated a gradual decrease in the titers by age 20, followed by an increase during their twenties (Fig. 1). Although other possibilities still remain to be excluded (e.g., puberty and other cohort factors) for influencing antibody titers of individuals aged 10 to 19, our present result implies the possibility that, due to the decline of otherwise protective immunity induced by the current vaccine, some of the vaccinees become susceptible again to infection with field strains of MV (secondary vaccine failure), which boosted their antibody responses. In this connection, Damien et al. estimated the susceptibility to asymptomatic secondary immune response against measles in vaccinated and late-convalescent persons to be 22.2 to 33.2% and 3.2 to 3.9%, respectively (7). In general, people in their late twenties have close contact with young children, the target population of MV, through taking care of their own children. It is reasonable, therefore, to assume that such individuals with marginal levels of anti-MV immunity can be infected with MV although they may not necessarily exhibit typical clinical symptoms. Since secondary vaccine failure could contribute to the occurrence of measles cases in an epidemic (16), revaccination of such individuals needs to be considered in order to sustain an antimeasles immune status (2).

Maternal NT antibodies became undetectable 8 months after birth in most infants (Fig. 1). Ohsaki et al. (17) observed that the average NT titers in cord blood has been gradually decreasing over the past few decades, due to the decreased opportunities for mothers-to-be for recurrent exposure to natural measles to maintain high antibody levels. If natural measles continues to decrease, the average titers of maternal NT antibodies will decrease further, resulting in a yet-earlier disappearance of maternal NT antibodies in infants. Considering the significant incidence of measles among infants within a year after birth (17% of total cases in Osaka in 1999-2000), these observations might suggest administering the vaccination earlier than the current recommended age of between 1 and 2 years. The idea of early vaccination may be challenged by an argument that vaccination at such young infancy may not induce adequate levels of anti-MV immunity (9, 15). However, Stetler et al. (24) reported that more than 70% of children who received the first vaccination before 10 months of age were shown to have NT antibodies at 15 months or older and that 98% of children who received the second dose at this timing had measurable NT antibodies 8 months after revaccination. To improve measles control in countries and regions with a high disease burden, the World Health Organization recommends that the first dose be given at age 9 months, followed by the second dose after the first birthday. As for countries with a low disease burden, the United States currently recommends a vaccination program in which the first dose is given at age 12 to 15 months and the second dose before kindergarten entry, with the first dose coverage being more than 90%.

Tamin et al. (27) reported that field strain Chicago-1 (type D3) possessed a unique antigenic epitope and that sera obtained from naturally infected subjects neutralized the Chicago-1 strain with titers about five times higher than those of vaccine viruses (type A). On the other hand, Ward et al. (32) reported that the NT antibody titers of naturally infected individuals and vaccinees against Edmonston vaccine strain (type A) and wild-type Chicago-1 strain were in good correlation. Similarly, Klingele et al. (12) demonstrated that there was no significant difference in NT titers against the Chicago-1 strain between vaccinee sera and late-convalescent-phase sera, although some, but not all, field strains were rather resistant to neutralization by the vaccinee sera. In this connection, Kobune et al. (13) demonstrated a small but distinct difference in an-

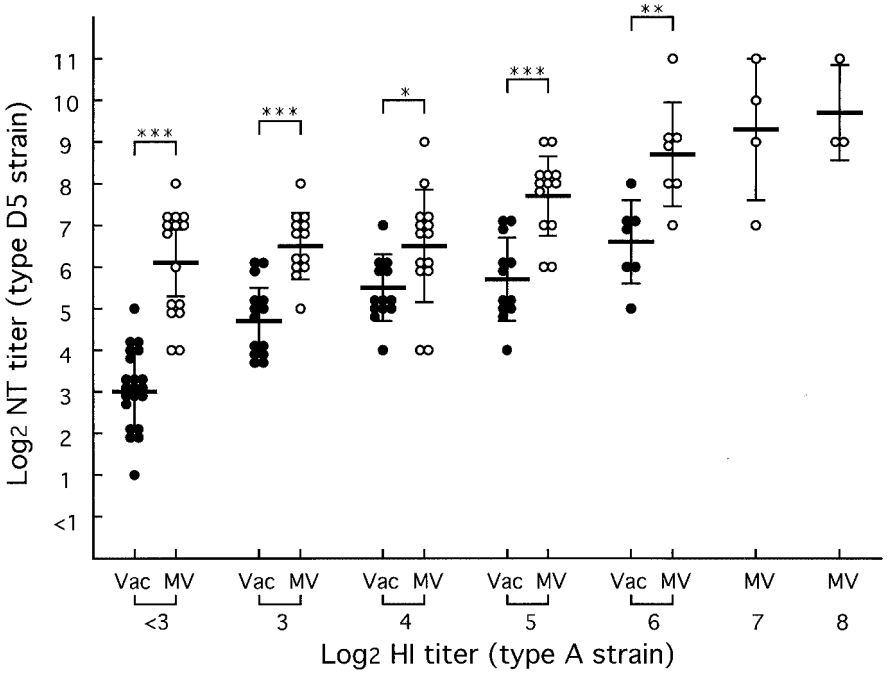

FIG. 4. Comparison of NT antibody titers between vaccinees and naturally infected individuals with matched HI titers. Thick and thin bars represent mean titers and standard deviations, respectively. ●, vaccinees under 19 years old; ○, naturally infected subjects from all age groups; *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$ (Student's t test).

tigenicity between MV isolates obtained from the same throat swab using B95a and Vero cells. It is thus possible that a difference(s) in viral antigenicity generated through different isolation procedures and/or passage history accounts for the reported discrepancy using the same strain, Chicago-1. Our present results demonstrated that sera obtained from vaccinees and naturally infected individuals neutralized the vaccine strain (type A) and field strains (types D3 and D5) with practically the same titers (Fig. 2; also data not shown). Our observation is also consistent with a report by a Japanese research group (H. Sakai et al., Report on the effective implementation of vaccination and its adverse effects; research project on adverse effects of vaccination, 1998-2001; Ministry of Health, Labour and Welfare, Tokyo, Japan). Tamin et al. (27) described in their report that the contribution of antigenic changes to the epidemiology of MV infections was still unclear and that the recent field strains were still neutralized by vaccine-induced antibodies. Taken together, these results support the idea that the current vaccine strains would be suitable to elicit protection against the D3 and D5 genotypes, as long as viral antigenicity is concerned.

The comparative analysis of NT and HI titers suggests a qualitative difference, in addition to the quantitative difference (Fig. 1), between anti-MV antibodies induced by vaccination and those induced by natural infection (Fig. 4). The observed difference is unlikely to be attributed to different viral antigenicities between the viruses used in NT and HI tests, since sera of vaccinees and naturally infected individuals reacted equally to both the vaccine and field strains (Fig. 2). Neutralizing and protective epitopes have been identified not only on the H protein but also on the F protein of MV (1, 8), and antibodies against such epitopes can be detected in human sera after acute MV infection (1). In general, antibodies induced under proper conditions, including repeated immunization, become more mature and have stronger affinity to the target antigens (affinity maturation). In monkey models, MV field strains replicate more strongly and efficiently to induce stronger and longer-lasting immune responses than the vaccine strains (14, 31). It thus appears that anti-MV immunity is more effective and lasts longer in naturally infected humans than in vaccinees. It would be interesting to see whether or not repeated vaccination can induce more-affinity-mature, longer-lasting antibodies.

In conclusion, anti-MV antibodies induced by vaccination were shown to be less capable of neutralizing viral infectivity than those induced by natural infection and to decline significantly over decades. It should be emphasized that vaccine-induced protective immunity is not necessarily lifelong without boosting. To fully control measles in Japan, implementation of a better vaccination program, such as a two-dose vaccination schedule with high vaccination coverage at an earlier time in childhood, should be considered.

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