A SEROSURVEY TO IDENTIFY THE WINDOW OF VULNERABILITY TO WILD-TYPE MEASLES AMONG INFANTS IN RURAL MALI

MILAGRITOS D. TAPIA,* SAMBA O. SOW, SANDRA MEDINA-MORENO, YU LIM, MARCELA F. PASETTI, KAREN KOTLOFF, AND MYRON M. LEVINE
Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland: Centre pour le Développement des Vaccins-Mali, Bamako, Mali

Abstract. As infants lose maternally derived antibody, they experience a period when antibody levels are insufficient to protect against measles yet may interfere with immunization. In Kangaba Mali, sera were collected from 89 2–8-month-old infants and 32 9–10-month-old infants without a history of measles or vaccination; post-vaccination sera were collected from 24 of the 9–10-month-old infants 3–5 weeks after receiving measles vaccine. Measles antibody was measured by plaque reduction neutralization (PRN) and enzyme linked immunosorbent assay. At two months of age, 30% had protective PRN titers; among six-month-old infants, none had protective titers. Prior to vaccination, 16% of 9–10-month-old infants exhibited protective titers; all demonstrated protective titers post-vaccination. The early onset of the window of vulnerability in Kangaba infants likely reflects the changing ecology of measles in Africa. Ways to protect these vulnerable infants against measles must be devised.

INTRODUCTION

Despite the availability of an inexpensive and highly effective attenuated vaccine, in 2000, the World Health Organization (WHO) estimated that measles caused 770,000 deaths, most of which occurred in Africa. A notable proportion of cases and deaths occur in infants younger than nine months of age, before reaching the age at which measles vaccination is currently recommended in developing countries. Several factors conspire to allow measles to persist as a public health problem, particularly in the developing world. First, routine vaccine coverage of infants is low, especially in remote rural districts and urban slums where access to immunization services is often impeded. Second, the current parenteral attenuated vaccine does not elicit high antibody levels when administered to infants less than nine months of age. Finally, while maternal antibodies at adequate titer can protect infants early in life, multiple factors influence the quantity and quality of antibodies that cross the placenta. As a result, many young infants experience several months during which the titer of maternal antibodies falls below a level that can protect them against wild-type measles virus, but may interfere with antibody production in response to attenuated measles vaccine virus. During this window of vulnerability, young infants may develop severe or fatal measles if exposed to wild-type virus. In the early 1990s, vaccination of six-month-old infants with high titer measles vaccine was explored as a strategy to overcome maternal antibodies and enable successful immunization of infants during this window. However, excess mortality among female infants led to abandonment of this approach.

The primary strategy now being pursued to control the measles disease and mortality burden in developing countries, including in young infants, is based on mass immunization campaigns that target older infants and children with current vaccines. By drastically diminishing the incidence in this population, it is hoped that young infants will be indirectly protected. In Mali, where during the early 1990s approximately 231,000 measles cases and approximately 13,850 deaths occurred annually, the Ministry of Health of Mali and the WHO in 1998 and 1999 organized mass immunization campaigns with parenteral measles vaccine for urban children 9–59 months of age. These campaigns, which were carried out in association with National Immunization Days performed as part of the Polio Eradication Initiative, diminished the incidence of measles by 95% in vaccinated areas. Another measles vaccination campaign conducted in 2001 included children up to 14 years of age, and a national campaign conducted in late 2004 targeted children 9–59 months of age. While effective, such campaigns are expensive and temporarily tie up many immunization service resources.

An adjunct control strategy being pursued aims to help by immunizing with a new generation of measles vaccine infants in developing countries who are less than six months of age. In support of this strategy, the Bill and Melinda Gates Foundation has sponsored initiatives to develop a measles vaccine that can be safely administered to young infants during the window of vulnerability. The new vaccines may supplement what can be accomplished with the current measles vaccines.

Information on the prevalence and magnitude of neutralizing antibody titers at different time points during infancy are needed to help guide the development of these new vaccines and to monitor the impact of mass immunization campaigns on the serologic status of infants. Notably, the most recent serosurveys to study the window of vulnerability in developing countries using the gold standard plaque reduction neutralization (PRN) assay were conducted in the 1980s and 1990s when most mothers had antibodies from natural infection rather than vaccination and when the incidence of measles was higher. To elucidate the window of vulnerability among infants in Kangaba, Mali and to better understand the changing epidemiology of measles, we performed a PRN assay serosurvey of infants living in a typical rural village setting in the Koulikoro region. We also used this opportunity to evaluate, in parallel, a simpler enzyme-linked immunosorbent assay (ELISA) for IgG antibodies to measles virus.

* Address correspondence to Milagritos D. Tapia, Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore Street, Room 480, Baltimore, MD 21201. E-mail: mtapia@medicine.umaryland.edu

MATERIALS AND METHODS

Study site. Mali, a land-locked west African country, is divided into nine administrative regions containing 58 cercles
Briefly, 96-well plates were prepared by the Applied Immunology Unit at the Center for Vaccine Development (CVD) in Baltimore, Maryland for analysis; the second aliquot remained at CVD-Mali. Serum samples were frozen and thawed no more than three times.

Plaque reduction neutralization assay. Serum samples were incubated with 100 plaque-forming units of wild-type (Edmonston strain) measles virus for one hour at 37°C in an atmosphere of 5% CO₂ and then plated onto confluent (-90% density) monolayers of Vero cells (American Type Culture Collection, Manassas, VA) in 12-well plates, in an adaptation of the method of Albrecht and others.25 After incubation for one hour, the serum-virus mixture was removed and the cells were overlaid with 2× minimum essential medium with 2% fetal bovine serum and Sea Plaque Agarose (Cambrex Bio Science Rockland Inc, Rockland, ME) (2 mL/well) and incubated for five days. Wells were stained with neutral red (Gibco Invitrogen Corp., Grand Island, NY), incubated overnight, and the number of plaques in each well was counted. International standard serum (66/202) from the WHO was measured in parallel with the samples, thereby permitting expression of PRN antibody in miU/mL. Samples with undetectable neutralizing antibody (titer < 10 by PRN) were arbitrarily assigned a value of 6.25 miU/mL, the equivalent of a titer of 5 by PRN. Titers ≥ 200 miU/mL were considered protective.27–29

Measles-specific IgG ELISA. Briefly, 96-well plates were coated with measles virus lysate (Advanced Biotecnologies Inc, Columbia, MD) at a concentration of 5 μg/mL in carbonate buffer, pH 9.0, for three hours at 37°C, and blocked overnight with 10% milk (Nestle USA Inc., Glendale, CA) in phosphate-buffered saline (PBS). After incubation, plates were washed 6 times with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBST). Serum samples were tested in two-fold dilutions in 10% dried milk in PBST (PBSTM). Plates were incubated for one hour at 37°C and washed as described above. Specific IgG against measles virus was detected with peroxidase-labeled goat anti-human Fc γ chains (ICN, Irvine, CA) diluted 1:5,000 in PBSTM. The secondary antibodies were incubated for one hour at 37°C. The substrate solution used was tetramethylbenzidine microwell peroxidase (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD). After incubation for 15 minutes in the dark, the reaction was stopped by the addition of 100 μL of 1 M H₂PO₄ and the optical density at 450 nm OD₄₅₀ was measured in an ELISA micro plate reader (Multiskan Ascent; Thermo Labsystems, Franklin, MA). Sera were run in duplicate and negative and positive control sera were included in each assay. Linear regression curves were plotted for each serum sample and titers were calculated (through equation parameters) as the inverse of the serum dilution that produces an OD of 0.2 above the blank (ELISA units/mL). Samples with < 5 ELISA units/mL were considered to have undetectable levels of antibody. Measles-specific titers were also expressed in miU/mL by interpolating regression-corrected OD values of serum samples in the curve of the WHO standard for anti-measles serum 66/202.30 The limit of sensitivity of the assay was 5 ELISA units/mL or 0.20 miU/mL.

Data analysis. Geometric mean titers (GMTs) of serum PRN antibody and measles-specific IgG were calculated for each age group. In addition, the proportion of infants in each
age group with a neutralizing antibody titer \( \geq 200 \text{ mIU/mL} \) (considered to be the protective level) were calculated. Titters measured by PRN and ELISA were log-transformed and the correlation coefficient was calculated using Small Stata 8 (Stata Corporation, College Station, TX).

**RESULTS**

A total of 149 serum samples were collected from 125 2–10-month-old infants; 52% were males. All participants provided a baseline blood sample. Pre-vaccination and post-vaccination samples were obtained from 24 (75%) of the 32 9–10-month-old infants before and 21–24 days after measles vaccination. During routine Good Clinical Practice quality control of case report forms, errors in calculation of age were noted for three infants who had blood samples taken; since they did not meet the inclusion criterion, they are excluded from analysis. One infant’s serum sample was not shipped to Baltimore for analysis.

Titters of PRN antibody by age are shown in Table 1 and presented graphically in Figure 1. Among two-month-old infants, the youngest age group tested, 95% had detectable antibody but only 30% exhibited protective titers (\( \geq 200 \text{ mIU/mL} \)). Similarly, whereas 90% of the four-month-old infants had detectable antibody, only three subjects (15%) had a protective titer (with two being convincingly above the protective threshold). By age six months, none of the 30 infants exhibited protective titers; moreover, < 40% had detectable antibody. This age group manifested the GMT nadir of PRN. Among 9–10-month-old infants prior to vaccination with measles vaccine, 50% had detectable antibody and approximately 16% already exhibited protective titers of measles PRN antibody. Notably, one month following administration of measles vaccine, 100% of subjects reached protective titters.

Antibody titters measured by IgG ELISA are shown in Table 1 and Figure 2. The age-specific seroepidemiologic curve of IgG ELISA antibody (Figure 2) closely paralleled that of PRN antibody. Results obtained by ELISA correlated well with the PRN assay results (r = 0.93, P < 0.001) and were reproducible between runs (coefficient of variation < 5%, r = 0.93, P < 0.001). The ELISA had a sensitivity of 100% in being able to detect antibody in all infants who had detectable PRN titers. Among all infants who had PRN titers \( \geq 200 \text{ mIU/mL} \), 87% also had measles-specific IgG levels \( \geq 200 \text{ mIU/mL} \) measured by the ELISA. All infants with less than protective levels of neutralizing antibody (i.e., < 200 mIU/mL) had measles-specific IgG ELISA titers < 200 mIU/mL. The ELISA was less sensitive than PRN in detecting infants who reached protective levels one month post-vaccination as only 88% of the infants exhibited such levels by ELISA versus 100% by PRN.

**DISCUSSION**

The window of vulnerability to wild-type measles infection among infants in Kangaba, Mali, measured by PRN begins at two months of age and extends to nine months of age when it is recommended that the current measles vaccine should be administered in developing countries. There are few publications describing the seroepidemiology of measles during infancy in developing countries in recent years. Moreover, most published studies measured measles antibody by methods other than the gold standard PRN and were completed in the 1980s and 1990s when mothers were more likely to have measles antibodies derived from natural infection than from vaccination. In only two studies, one in the Congo and the other in Bangladesh, was neutralizing antibody measured, albeit by different techniques. Although

**TABLE 1**

Titer of measles antibody by PRN assay and proportion with PRN titers in the protective range

<table>
<thead>
<tr>
<th>Infant age (months)</th>
<th>No.</th>
<th>% with detectable antibody† (95% CI)</th>
<th>GMT by PRN (mIU/mL)</th>
<th>Range (mIU/mL)</th>
<th>% with PRN ( \geq 200 \text{ mIU/mL} )‡ (95% CI)</th>
<th>GMT by ELISA (mIU/mL)</th>
<th>Range (mIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>20</td>
<td>95 (75–100)</td>
<td>85</td>
<td>6–684</td>
<td>30 (12–54)</td>
<td>67</td>
<td>6–362</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>90 (68–99)</td>
<td>55</td>
<td>6–1,464</td>
<td>15 (3–38)</td>
<td>22</td>
<td>2–341</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>36.7 (20–56)</td>
<td>10</td>
<td>6–40</td>
<td>0 (0–12)</td>
<td>7</td>
<td>1–41</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>52.6 (29–76)</td>
<td>16</td>
<td>6–183</td>
<td>0 (0–18)</td>
<td>9</td>
<td>2–170</td>
</tr>
<tr>
<td>Pre-vaccination§</td>
<td>32</td>
<td>50 (32–68)</td>
<td>20</td>
<td>6–2,926</td>
<td>15.6 (5–33)</td>
<td>12</td>
<td>2–1,690</td>
</tr>
<tr>
<td>Post-vaccination§</td>
<td>24</td>
<td>100 (86–100)</td>
<td>2,997</td>
<td>375–18,074</td>
<td>100 (86–100)</td>
<td>805</td>
<td>141–3,146</td>
</tr>
<tr>
<td>Total</td>
<td>121</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* PRN = plaque reduction neutralization; CI = confidence interval; GMT = geometric mean titer; ELISA = enzyme-linked immunosorbent assay.
† Neutralizing antibody level > 6.25 mIU/mL.
‡ Protective level.22,28
§ Refers to receipt of measles vaccine. Infants in the pre-vaccination group were 9–10 months old and those in the post-vaccination group were 10–11 months old.

![Figure 1. Scatterplot of plaque reduction neutralizing (PRN) antibody titters and geometric mean titers (GMT) measured in different age groups of Malian infants. The horizontal line represents the cutoff for protection (200 mIU/mL). Ages are in months. Pre = pre-vaccination; Post = post-vaccination.](image-url)
one must be cautious in comparing results from studies in different geographic areas that used different techniques to measure neutralizing antibody, some intriguing differences are noted. In 1986 in Brazzaville, 19% of the six-month-old infants tested had antibody titers > 40 mIU/mL, whereas in Kangaba, no six-month-old infant participant had a titer in this range. While in Bangladesh in the 1990s > 90% of the two-month-old infants had protective levels, in Kangaba only 30% of the two-month-old infants had titers in the protective range.

These data suggest that during pregnancy mothers in Kangaba may be transferring across the placenta only a small amount of measles antibody relative to that observed in the past in developing countries. Previous studies have demonstrated that placental transfer of antibodies in women of developing countries is lower when compared with industrialized countries. While HIV infection is recognized to reduce maternal antibody transfer, this is unlikely to be the responsible factor in this setting because of the low prevalence of HIV infection (< 2%). Placental malaria infection, which can reduce placental transfer, may be a contributing factor given that malaria is holoendemic in Mali. However, the most likely explanation for the expansion of the window of vulnerability observed in Kangaba is that the epidemiology of measles is changing such that antibody levels in these mothers are decreased relative to those found 10–20 years ago in developing countries. Given the advances in measles control, our findings were to be expected, but had not yet been described. In 1986, routine measles vaccination was introduced in Mali for infants at nine months of age and campaigns including children nine months to six years of age were conducted. Although coverage has been spotty, a sizable proportion of women of child-bearing age now have vaccine-induced levels of measles antibody that are lower than wild-type infection-induced levels. Infants born to vaccinated mothers would therefore receive less antibody by placental transfer. Also, if there is less exposure to wild-type measles than in past generations, mothers may not experience boosting of their measles antibody and their infants may not benefit from high levels of antibody. Boosting from wild-type virus will decrease even further if follow-up mass immunization campaigns are carried out every few years. Consequently, we conclude that infants are not receiving large amounts of antibody transplacentally and may be more vulnerable to wild-type measles infection at an earlier age than previously observed. As further expansion of the window would be expected, this trend should be monitored in other areas of Mali, as well as in other African and Asian countries to document serologically the change in the ecology of measles virus and the impact of this change on the susceptibility of young infants.

The window of vulnerability age span comprises a heterogeneous population of infants, where many infants may have low or undetectable antibody levels, leaving them at risk of clinical illness due to wild-type infection and others may have protective PRN levels. Because of this variability and the immaturity of the immune system, vaccination of six-month-old infants with standard dose vaccine is not recommended except during epidemics. While the standard vaccine induces T cell responses in infants as young as six months of age, strategies for some vaccines under development are aiming to vaccinate even younger infants so that protection afforded by maternal antibodies may be bridged with that induced by vaccine. Our data provide invaluable information for the design of future vaccine clinical trials and suggest that in Mali a measles vaccine is needed that is safe and can induce antibody responses in infants as young as two months of age.

The immune response that best correlates with protection from measles is the presence of neutralizing antibodies. The PRN assay is considered the gold standard in measuring neu-
ralizing antibodies and a titer ≥ 120 was associated with protection from disease. With the aid of a WHO standard serum, investigators can quantify neutralizing antibodies in mIU/mL, thereby permitting comparison of results among laboratories. Since the PRN assay is a rigorous, expensive and time-consuming test, a simpler, sensitive, and more economical serologic test would be more appropriate for use in developing country laboratories. A practical alternative would be the ELISA, if the assay is sufficiently sensitive and specific and correlates well with PRN. Several ELISA kits that measure measles antibody are available commercially. While these kits are easy to use, they are generally not sufficiently sensitive to detect low levels of antibody and are therefore not optimal for seroprevalence surveys such as the one described herein.41,42

The ELISA that we developed for use in this serosurvey proved to be 100% sensitive in detecting measles neutralizing antibodies relative to the PRN and there was a significant correlation between the two assays (r = 0.93). At a level of 200 mIU/mL, the ELISA had a sensitivity of 87% and a specificity of 100% in identifying individuals who had protective titers of neutralizing antibody measured by PRN. The ELISA was able to quantify different levels of antibody among those with PRN titers < 20, supporting the ability of this assay to measure low levels of antibody. Lastly, 88% of all vaccinated infants had a titer > 200mIU/mL, demonstrating that this assay is able to identify appropriate immune responses to measles vaccination. Although this ELISA is not available as a commercial kit, several large lots of measles antigen from a commercial source have given repeatable and consistent results. These attributes support the notion that this ELISA could be established in developing country laboratory settings for use with seroepidemiologic surveys and measurement of vaccine-induced immune responses.

Received October 8, 2004. Accepted for publication December 22, 2004.

Acknowledgments: We thank Dr. Mama Niele Doumbia and Dr. Modibo Bagayogo, as well as the staff at the Kangaba and Salamalé Centres de Santé Communauté, especially Dr. Koli Sissoko, for their assistance in completing this study.

Financial support: This study was supported by a grant from the Bill and Melinda Gates Foundation (Myron M. Levine, Principal Investigator).

Authors’ addresses: Milagritos D. Tapia, Sandra Medina-Moreno, Yu Lim, Marcela F. Pasetti, Karen Kotloff, and Myron M. Levine, Center for Vaccine Development, University of Maryland School of Medicine, 685 West Baltimore Street, Room 480, Baltimore, MD 21201, Telephone: 410-706-5328, Fax: 410-706-6205, E-mails: mtapia@medicina.umd.edu, kmoren@medicina.umd.edu, ylim@medicina.umd.edu, mpasetti@medicina.umd.edu, kktolof@medicina.umd.edu, and mlevine@medicine.umd.edu. Samba O. Sow, Centre pour le Développement des Vaccins—Mali, Centre National d’Appui à la Lutte contre la Maladie, BP 251, Bamako, Mali, Telephone/Fax: 223-223-60-31, E-mail: ssw@medicine.umd.edu.

REFERENCES


