Comparison of Immunoglobulin G Subclass Profiles Induced by Measles Virus in Vaccinated and Naturally Infected Individuals

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A total of 258 human sera positive for measles antibodies were divided into four different groups: group 1 contained 54 sera from children after natural measles infection (immunoglobulin M [IgM] positive, early infection phase), group 2 contained 28 sera from children after measles vaccination (IgM positive, early infection phase), group 3 contained 100 sera from healthy adults (natural long-lasting immunity), and group 4 contained 76 sera from healthy children (postvaccinal long-lasting immunity). In the early phase of infection, the percent distributions of measles virus-specific IgG isotypes were similar between natural and postvaccinal immune responses. IgG1 and IgG4 were the dominant isotypes, with mean levels of detection of 100% (natural infection) and 100% (postvaccinal) for IgG1 and 96% (natural infection) and 92% (postvaccinal) for IgG4. In comparison, the IgG4 geometric mean titer (GMT) in the early phase of natural infection was significantly higher than the IgG4 GMT detected in the postvaccinal immune response (80 versus 13; 95% confidence interval). In the memory phase, IgG2 and IgG3 responses decreased significantly in both natural infection and postvaccinal groups, while IgG1 levels were maintained. In contrast, the IgG4 postvaccinal immune response decreased strongly in the memory phase, whereas IgG4 natural long-lasting immunity remained unchanged (9 versus 86%; P < 0.05). The results obtained suggest that IgG4 isotype could be used in the early phase of infection as a quantitative marker and in long-lasting immunity as a qualitative marker to differentiate between natural and postvaccinal immune responses.

Measles has been targeted for global eradication by the World Health Organization’s Expanded Programme of Immunization (4). For the effective control and eventual eradication of measles, it is necessary to impair measles transmission by establishing population immunity (5). In addition, laboratory and epidemiological studies should be conducted to address genetic and antigenic measles virus variability as well as measles virus-specific immune responses. Such studies should examine (i) genetic diversity between measles virus vaccine and wild-type strains to ensure that existing vaccines continue to provide a high degree of protection, (ii) the response to measles vaccine provided at different schedules of vaccination (ages and intervals), and (iii) serological markers at different stages of measles infection to globally understand antibody responses to the infection (12, 20, 21). Recently, a subclass-resistant response to antigens was demonstrated; however, limited data are available on measles virus-specific immunoglobulin G (IgG) subclass responses (11, 14, 16, 24). We have defined two highly different measles immune IgG isotypic response patterns which make it possible to differentiate convalescence phase and memory phase immune responses during natural measles infection. (13). The data reported support the hypothesis that the IgG isotypic immune response could be highly useful for the diagnosis and analysis of antibody responses to measles infection.

IgM antibody detection currently is effectively used to diagnose a primary measles infection. In addition, the detection of total measles virus antibodies is an indicator of long-lasting immunity. These serological markers do not differ between natural and postvaccinal responses. The present study was undertaken to compare the specific anti-measles IgG1, IgG2, IgG3, and IgG4 subclass response patterns elicited during natural and postvaccinal responses.

MATERIALS AND METHODS

Serum specimens. A total of 258 human serum samples positive for measles virus antibodies were used in this study. Serum specimens were classified into four groups according to the source of infection (natural measles infection and vaccination) and the phase of infection (recent or long-lasting immune response).

(i) Group 1. Group 1 consisted of 54 individuals (2 months to 44 years old; median age, 17 years old) from whom a single serum sample was obtained. The samples were obtained after natural measles infection during a measles virus outbreak in Argentina in 1998. All the samples showed the presence of measles virus-specific IgM; 32 of these (group 1a) were acute-phase serum samples obtained within 1 week after the onset of rash (median, 3 days; range, 1 to 7 days), and 22 (group 1b) were convalescent-phase serum samples obtained between days 8 and 26 after the onset of rash (median, 17 days).

(ii) Group 2. Group 2 consisted of 28 serum samples selected during a prospective study of adverse reactions to measles vaccine conducted during measles interepidemic periods in Argentina. These samples were obtained from 28 previously unvaccinated children (8 to 24 months old; mean age, 13 months old) who received the combined measles-mumps-rubella viral vaccine (MMR) or mono-valent measles vaccine according to the vaccine available at the time and who had an adverse reaction to the vaccine (mean fever, 37.6°C; mild rash occurring 7 to 18 days after measles vaccination). None of these children had an exanthematous
disease consistent with measles infection prior to the measles vaccination. The conditions mentioned above allowed us to confirm that the sera assayed were true postvaccination sera and to classify the samples obtained as acute- and convalescent-phase samples according to the day postrash on which they were collected. Fifteen out of 28 (group 2a) were postvaccinal acute-phase serum samples obtained within 1 week after the onset of rash (median, 3 days; range, 1 to 7 days), and 13 (group 2b) were convalescent-phase serum samples obtained between days 8 and 30 (median, 19 days). Measles infection was confirmed in group 1 and group 2 serum samples by the detection of measles virus-specific IgM antibodies by an immunoﬂuorescence assay (IFA) as a screening method and subsequently conﬁrmed by a capture enzyme immunoassay (method described below).

(iii) Group 3. Group 3 consisted of 100 serum samples obtained from healthy adults (40 to 85 years old; median age, 63 years old) who reported a history of long-pass natural measles infection at least 10 years earlier. The sera were collected at random from healthy patients undergoing routine physical examinations.

(iv) Group 4. Group 4 consisted of 76 serum samples selected from a large serum collection from a measles vaccine efficacy study conducted in a northern area of Cordoba Province, Argentina (San Alberto, San Javier, and Pocho; Sanitary Region 2) in 1994. These samples were from vaccinated healthy children who had received measles vaccine 8 to 12 years earlier (children vaccinated at between 11 and 15 months of age). All these children had documented measles vaccination (combined viral vaccine [MMR] or monovalent measles vaccine, according to the vaccine available at the time), and none of them had an exanthematos disease consistent with measles infection between the time of measles vaccine application and the day on which the serum samples were obtained. When the serum samples were obtained, the children ranged in age from 9 to 14 years old (median age, 10.8 years old). Only one serum sample was obtained from each child, and serum samples were stored at –20°C until they were processed. Past measles infection was conﬁrmed in group 3 and 4 serum samples by the detection of measles virus neutralizing antibody titers of ≥1:32 milli-international reference units (mIRU)/ml.

Antisera. Mouse monoclonal antibodies to human IgG subclasses were obtained from Sigma Chemical Co., St. Louis, Mo. These antibodies were used at dilutions of 1:100 (IgG1, 1:32; IgG2, 1:32; IgG3, and 1:32; IgG4) according to the manufacturer’s instructions. Rabbit monoclonal antibodies to human IgM and total IgG were obtained from Cappel and used at dilutions of 1:100 and 1:150, respectively. The optimal dilutions of monoclonal antibodies were determined by titrations against reference positive sera diluted 1:20 (initial dilution of sera for the IFA).

Preparation of antigen slides. A suspension of Vero cells (10⁶ cells/ml) was seeded in a 25-cm² bottle and infected with the Edmonston-Schwarz strain of measles virus at a multiplicity of infection of 0.1. Infected cells (50 µl) and uninfected cells (50 µl) were placed on each well slide. They were incubated for 48 h at 37°C in a humidified incubator with 5% CO₂. The monolayers were subsequently washed twice with phosphate-buffered saline (PBS [pH 7.2]) and once with distilled water. The ﬁxation of cells was done with acetone at 4°C for 10 min. Finally, the slides were stored at –20°C for later use.

IFA. For the IFA, in brief, twofold dilutions of serum samples were incubated with ﬁxed cells for 30 and 90 min for IgG and IgM antibody detection, respectively, at 37°C in a humididiﬁed chamber. The samples were then washed three times with PBS for 10 min per wash and incubated for 30 min at 37°C with ﬂuorescein isothiocyanate-conjugated anti-human IgM, total IgG, and IgG subclasses. After two washes with PBS for 10 min per wash, the slides were mounted with glycerol buffer on coverslips and then examined under a ﬂuorescence microscope at a ×40 objective magniﬁcation (20).

Fluorescence intensity may be considered semiquantitative on the basis of the guidelines established by the Centers for Disease Control and Prevention, Atlanta, Ga.: 4+, maximal ﬂuorescence, brilliant yellow-green; 3+, less brilliant yellow-green ﬂuorescence; 2+, deﬁnite but dull yellow-green ﬂuorescence; and 1+, very dim and subdued ﬂuorescence.

A serum dilution was considered positive for measles virus IgM and IgG antibodies if, in the presence of a ﬂuorescence intensity of 1+ or more, there was well-deﬁned staining of cytoplasmatic granules in cells coalescing to form multineucleated giant cells.

To deﬁne the IFA cutoff, a panel of true measles virus-negative IgG serum samples (reference technique, neutralization test) was assayed. Some negative serum samples at dilutions of <1:20 (<1:15 and 1:10) showed nonspeciﬁc reactions. Therefore, a dilution of 1:20 was deﬁned as the starting working serum dilution. A serum dilution was considered negative for measles virus IgM or IgG antibodies if the cells exhibited less than 1+ ﬂuorescence and displayed the reddish orange countetant or if the ﬂuorescence observed was not the speciﬁc staining pattern for measles.

IgM antibody capture enzyme immunoassay. The IgM antibody capture enzyme immunoassay for measles conﬁrmation was carried out at the National Reference Laboratory (Santa Fe, Argentina) as described by Erdman et al. (7).

Briefly, goat anti human IgM antibodies diluted in PBS were used to coat microtiter plates for 1 h at 37°C. After the plates were washed, serum diluted 1:100 in PBS was added to four consecutive wells, and the plates were incubated for 1 h at 37°C. After the plates were washed, either baculovirus-measles virus nucleoprotein or uninfected S9 cell culture lysate diluted in PBS-GT (0.5% gelatin and 0.15% Tween 20) with 4% normal goat serum was added to duplicate wells. The plates were then incubated for 2 h at 37°C and washed, biotinylated antibody to measles virus was added to each specimen, and the plates were incubated for 1 h at 37°C. The plates were washed three times, streptavidin-peroxidase was added, and the plates were incubated for 20 min at 37°C. Then, a solution containing tetramethylbenzidine and hydrogen peroxide was added, and the mixture was incubated for 15 min at room temperature. Color development was stopped by the addition of 1 N sulfuric acid solution, and the absorbance was read at 450 nm. For each sample, we calculated the difference between the mean optical density for the antigen-positive wells and the mean optical density for the negative control wells (difference, P – N values).

Seroneutralization assay. A seroneutralization assay was performed as described by Nates et al. (17). The highest dilution of serum that completely inhibited the cytopathic effect was recorded as the endpoint of antibody titration. Titer equals or higher than 1:2 were considered positive. A measles virus control and an international reference preparation of measles virus-specific antibody were used in every 96-well microculture plate and diluted in the same manner as the test samples. Results were converted to mIRU per milliliter of serum based on parallel assay results. Titer of ≥32 mIRU/ml were deﬁned as seropositive based on the test sensitivity at the ﬁrst serum dilution of 1:2.

Data analysis. Results are expressed with a 95% conﬁdence interval (CI), and the chi-square distribution test was used to analyze data.

RESULTS

A total of 258 human serum samples were studied by IFA for the detection of IgG1, IgG2, IgG3, and IgG4 measles virus-specific antibodies. The means and standard deviations (95% CI) for the seropositive measles virus-specific antibody isotypes from groups 1, 2, 3, and 4 are shown in Table 1. The measles virus-specific IgG1 subclass was detected at the acute phase in both naturally infected and vaccinated individuals

TABLE 1. Percent distributions of measles virus-specific IgG isotypes in the population studied

| Study group (source, phase of measles infection) | Time after onset of rash | Total no. of samples | Mean % (range) of isolates seropositive for:* |
|-----------------------------------------------|--------------------------|----------------------|-----------------------------------------------|
| 1a (natural, acute)                           | 0–7 days                 | 32                   | IgG1 94 (87–100) IgG2 34 (27–41) IgG3 44 (37–51) IgG4 50 (40–54) |
| 2a (postvaccinal, acute)                      | 0–7 days                 | 15                   | IgG1 100 (89–100) IgG2 13 (2–24) IgG3 40 (29–51) IgG4 68 (56–78) |
| 1b (natural, convalescence)                   | 8–26 days                | 22                   | IgG1 100 (91–100) IgG2 64 (55–73) IgG3 72 (63–81) IgG4 96 (87–100) |
| 2b (postvaccinal, convalescence)              | 8–30 days                | 13                   | IgG1 100 (88–100) IgG2 54 (42–66) IgG3 70 (58–82) IgG4 92 (80–100) |
| 3 (natural, long-lasting immunity)            | 10–80 years              | 100                  | IgG1 100 (94–100) IgG2 2 (0–8) IgG3 3 (0–9) IgG4 86 (80–92) |
| 4 (postvaccinal, long-lasting immunity)       | 8–12 years               | 76                   | IgG1 100 (93–100) IgG2 2 (0–9) IgG3 7 (0–13) IgG4 9 (2–16) |

* 95% CI.
and remained unmodified in long-lasting immunity. Similarly, no significant differences were found between IgG2 and IgG3 natural and postvaccinal immune responses \((P > 0.05)\). Thus, the daily percentages of IgG2 and IgG3 seropositivity gradually increased, reaching means of 64\% (group 1b) and 54\% (group 2b) and of 72\% (group 1b) and 70\% (group 2b), respectively, in the convalescence phase. The percentages of IgG2 and IgG3 seropositivity dropped significantly in the memory phase: 2\% (group 3) and 2\% (group 4) for IgG2 and 3\% (group 3) and 7\% (group 4) for IgG3. No significant differences between natural and postvaccinal responses \((P > 0.05)\) were observed in the percent distributions of the measles virus-specific IgG4 subclass in the acute and convalescence phases. In contrast, the IgG4 postvaccinal immune response dropped strongly in the memory phase, but IgG4 natural long-lasting immunity remained unchanged (9 versus 86\%) \((P < 0.05)\) (Fig. 1). Thus, the postvaccinal memory immune response was represented only by the IgG1 immune response. In addition, the IgG4 GMTs in natural measles infection were significantly higher than those detected in the postvaccinal response (80 versus 13) \((P < 0.05)\) in both the acute and the convalescence phases (early phase of infection) (Fig. 1). In contrast, the IgG1 GMTs were high in both natural and postvaccinal immune responses \((\geq 320)\). \((P > 0.05)\).

**DISCUSSION**

The response to measles virus at the IgG subclass level had not been previously characterized for vaccinated children; therefore, no measles data exist with which our results may be matched. Thus, our findings could be compared only to earlier investigations of antibody responses following varicella-zoster virus and hepatitis B virus infections. Our results indicated that in the early phase of infection, the percent distributions of measles virus-specific IgG4 isotypes are similar between natural and postvaccinal immune responses. Both responses showed the same isotype profile: in the acute phase, the IgG1 response was seen first, followed by IgG2, IgG3, and IgG4 responses, which increased gradually during convalescence. IgG1 and IgG4 were the dominant isotypes, reaching a high percent detection of 100 and 94\%, respectively. Gregorek et al. (9) reported that for convalescent-phase sera from naturally infected children, anti-HBs antibodies were highly restricted to...
IgG1 and IgG3, while IgG2 and IgG4 subclasses did not play significant roles. Similarly, in children immunized with recombinant HBsAg, IgG1 and IgG3 isotypes were predominant until 1 month after the vaccination schedule was completed. Therefore, in agreement with our results, the profile of anti-HBs antibodies in vaccinated children was very similar to that found after natural seroconversion. However, by comparison, our results showed that the measles virus IgG4 GMT in natural infection was significantly higher than the IgG4 GMT detected in the postvaccinal immune response (80 [95% CI, 33 to 191] versus 13 [95% CI, 7 to 26]). Thus, it is possible to establish a significant association between IgG4 antibody titers and probable exposure to measles vaccine or wild-type measles virus so that IgG4 titers could be used as a quantitative marker to differentiate the source of measles infection. In other words, IgG4 antibody titers of $\leq 30$ determined by an IFA could be designated to represent a vaccinal source of immunity and those of $>30$ could be designated to represent a natural infection. This study was conducted with vaccinated children who displayed an adverse reaction to the vaccine (group 2) because during epidemics of measles, it is necessary to distinguish between an adverse vaccine reaction and a natural measles infection.

In the memory phase, IgG2 and IgG3 responses decreased significantly in both natural infection and postvaccinal groups, while the IgG1 levels were maintained thereafter. The most interesting findings in our study were the high frequency and percent contributions of specific IgG4 to the total IgG anti-measles response in natural versus postvaccinal long-lasting immunity (86 versus 8.7%). These findings are supported by the results of Asano et al. (1), who found high levels of IgG1 and IgG4 antibody activity to varicella-zoster virus in the memory phase in the natural infection. These authors also reported that IgG2 and IgG3 isotypes could not be detected 10 years after varicella-zoster virus infection. However, Asano et al. (1) reported that the pattern of subclass-specific antibody response induced by the vaccine was almost equal to that seen after natural infection. In contrast, our findings defined a characteristic subclass restriction pattern to differentiate the source of measles infection in life-long immunity.

The regulation of antibody subclass expression in humans is not well understood. There is evidence that isotype switch recombination is a highly regulated process controlled by soluble cytokines and by T-cell membrane interaction regulation with the CD40 molecule on the B-cell surface (2, 3, 8, 15, 18). The regulation of antibody subclass expression in humans is not well understood. There is evidence that isotype switch recombination is a highly regulated process controlled by soluble cytokines and by T-cell membrane interaction regulation with the CD40 molecule on the B-cell surface (2, 3, 8, 15, 18). In contrast, whereas the CD40 molecule on the B-cell surface. In contrast, whereas the CD40 molecule on the B-cell surface.

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