Immunosorbent Assay Based on Recombinant Hemagglutinin Protein Produced in a High-Efficiency Mammalian Expression System for Surveillance of Measles Immunity

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Recombinant hemagglutinin (H) protein of the measles virus (MV) was produced in mammalian cells with a high-yield expression system based on the Semliki Forest virus replicon. Crude membrane preparations of H protein-transfected BHK-21 cells were used to coat microtiter plates to measure specific immunoglobulin G antibodies in 228 serologically defined serum samples mainly from measles late-convalescent adults. The titers by the enzyme-linked immunosorbent assay for the H protein (H-ELISA) closely correlated with neutralization test (NT) titers ($R^2 = 0.66$), hemagglutination inhibition test (HI) titers ($R^2 = 0.64$), with the titers from a certified commercial ELISA based on whole MV-infected cells (MV-ELISA; $R^2 = 0.45$). The correlations described above were better than those of the commercial MV-ELISA titers with the NT ($R^2 = 0.52$) or HI ($R^2 = 0.48$) titers. By using the 2nd International Standard for anti-measles serum, the detection level of the assay corresponds to 215 mIU/ml for undiluted serum, which corresponds to the estimated threshold for protective immunity. The specificity, accuracy, and positive predictive value were, in general, better for the H-ELISA than for a commercial MV-ELISA, independent of whether HI, NT, or HI and NT were used as “gold standards.” In contrast, the H-ELISA proved to be slightly less sensitive than the MV-ELISA (sensitivities, 98.6 versus 99.5%, respectively; $P$ was not significant). The assays did not differ significantly in the number of serum samples with positive HI and NT results ($n = 212$) which measured false negative (H-ELISA, 2 of 212 [0.94%]; MV-ELISA, 1 of 212 [0.47%]), but the H-ELISA detected significantly more measles-susceptible individuals than the MV-ELISA (10 of 11 versus 3 of 11, respectively; $P < 0.05$) among the individuals whose sera had negative HI and NT results. Our data demonstrate that the H-protein preparation that we describe could be a cost-effective alternative to current whole-virus-based ELISAs for surveillance for immunity to measles and that such an assay could be more efficient in detecting susceptibility to measles. Furthermore, unlike whole MV-based antigens, H-protein would also be suitable for use in the development of a simple field test for the diagnosis of measles.

Immunosorbent assay (ELISA) based on recombinant proteins may offer a number of advantages over whole-virus-based ELISAs. Such advantages include simplified production, improved standardization, and enhanced stability. An inexpensive and simple diagnostic test as an alternative to an MV-infected cell- or whole-virus-based ELISA is also required to monitor measles immunity as part of eradication programs (22). Such assays could rely on the detection of antibodies directed against selected MV proteins (48, 49). Antibodies against the nucleoprotein were found to correlate with total MV antibodies (27). Whether antibodies specific for other MV proteins also correlate with total MV antibodies and with immunity has not been demonstrated with a panel of human sera. Most functional antibodies are directed against the hemagglutinin (H) protein: they neutralize MV in vitro and provide protection against MV in vivo (9, 10, 14, 17, 20, 21, 32, 46). Therefore, H protein-specific immunoglobulin G (IgG) antibodies are considered to be most important in determining immunity to MV (6).

The MV H protein has been expressed in a number of expression systems including baculovirus (40, 47), vaccinia virus (14, 41, 51), canarypox virus (42), adenovirus (3), and other (19) systems. Expression of this glycoprotein in prokaryote or lower eucaryote systems should result in glycosylation different from that in MV. Proper glycosylation has been found to be important for the processing, the functional integrity, and the antigenicity of this protein (23–25). For proper posttranslational modification, this glycoprotein should therefore be expressed in mammalian cells. However, in most mammalian systems the yield is low (19). We analyze here whether the H protein expressed in a high-yield mammalian expression system based on the Semliki Forest virus replicon (29, 30) is suitable for monitoring measles immunity.
MATERIALS AND METHODS

Serum panel. Sera were obtained from 217 consecutive outpatients over the age of 25 years at the Laboratoire National de Santé who underwent venipuncture for measles-unrelated reasons in December 1995 and January 1996. The volunteers consisted of 87 males (age range, 25 to 80 years) and 130 females (age range, 25 to 92 years). It can be assumed that the vast majority of the persons in this age bracket had measles during their childhoods because they were born at a time (1905 to 1970) when immunity was mostly acquired by early natural infection. In addition, 11 negative serum samples were obtained from seven unvaccinated 15-month-old children, two adolescents, and two adults.

The hemagglutination inhibition test (HI) and neutralization test (NT) titers of the test sera were determined as described before (26). Titers are expressed as log dilutions, with values of ≤1.25 being negative. Anti-MV antibody levels were measured by using a certified commercial ELISA based on MV-infected simian cells, following the supplier's instructions.

The recombinant MV H protein. Three overlapping cDNA fragments of the MV H protein were obtained by reverse transcription-PCR from total RNA of virus-infected Vero cells. After appropriate digestions, the full-length 1.9-kb cDNA was reconstituted from these fragments into the BamHI cloning site of the pUC-18 vector (pUC-MVHrev), which was then transferred into the BamHI site of the pSFV1 plasmid (pSFV1-MVH, unpublished data). As described by Erkrath, Germany). The coefficient of determination \( \left( R^2 \right) \) was obtained by regression analysis. The characteristics of the assays (see Table 1) were calculated according to the definitions of Bland (7).

RESULTS

Detection of MV-specific IgG. The ability of our H-ELISA to detect MV-H-specific human IgG was tested with a panel of serologically characterized sera obtained from measles late-life adulthood. The comparison of the recombinant H-ELISA with the commercial MV-ELISA with all 228 serum samples gave an \( R^2 \) value of 0.45 (data not shown). Figure 1 shows the correlation of the titers obtained by the recombinant H-ELISA and of a certified commercial MV-ELISA with HI and NT titers. The better correlations with HI \( (R^2 = 0.64 \) versus 0.48) and NT \( (R^2 = 0.66 \) versus 0.52) titers were found with the recombinant H-protein assay. Readout values of H-ELISA after 60 or 90 min had identical \( R^2 \) values. The \( R^2 \) values for the H-ELISA presented above correspond to the \( R^2 \) for these sera obtained by NT and HI \( (R^2 = 0.67) \).

Analysis of false-positive and false-negative sera. Negativity and positivity were defined on the basis of the mODs (mean ± standard deviation [SD] = 54 ± 26) for the sera negative by both HI and NT (titers, ≤1:2). Sera with mODs of >120 (mean ± 2.5 SD) and mODs of <80 (mean ± 1 SD) were considered positive and negative, respectively, with 80 to 120 mOD units being a gray zone in which sera were undefined. The corresponding values for the commercial MV-ELISA are defined by the supplier as <100 and >200 mOD units. By these criteria, significantly more sera negative by both HI and NT were false positive by the MV-ELISA (6 of 11) than by the H-ELISA (0 of 11; \( P < 0.05 \) Fig. 2). Figure 2 also shows that among sera positive by both HI and NT the H-ELISA detected as many false-negative sera as the MV-ELISA (2 of 212 versus 1 of 212; \( P > 0.05 \) not significant). The HI and NT values for the false-negative sera were low (<1:26; cf. Fig. 2).

Since HI is thought to be less sensitive than NT (2, 34, 50), the analysis based on the titers obtained by HI and NT described above potentially excludes weakly positive sera. Figure 3 presents the HI and NT values for sera positive or negative by one of the two ELISAs. Of the sera which were negative by H-ELISA, 10 of 13 were negative by both HI and NT (Fig. 3A). All sera which were positive by H-ELISA were also positive by NT, with 4 of 213 serum samples being HI negative and NT positive and 209 of 213 serum samples being both HI and NT positive (Fig. 3B). Four serum samples were negative by the MV-ELISA; three of these were negative by both HI and NT (Fig. 3C). Among the sera positive by MV-ELISA, 6 of 222 serum samples were negative by both HI and NT, an additional 5 serum samples were HI negative and NT positive, and all others (i.e., 211 of 222) were positive by both HI and NT (Fig. 3D). A total of 209 of 213 (98.1%) and 211 of 222 (95.0%) of the serum samples which were seropositive by the H-ELISA or the MV-ELISA were positive by both HI and NT 10 of 13 (76.9%) and 3 of 4 (75.0%) of the serum samples which were negative by the H-ELISA or the MV-ELISA were negative by both HI and NT.

Performance of the H-ELISA. The different performance parameters of the H-ELISA were compared with those of whole-virus-based ELISAs from this study and from the literature (8, 12, 34). For a valid comparison, all values were re-calculated with the same algorithms by adding undefined sera to the sera negative by ELISA (7). The results of the latter comparison are presented in Table 1. The performance of the H-ELISA compared favorably with those of the whole-virus ELISAs both when results for the same or different cohorts were considered. Results were similar whether undefined sera were excluded or added to the negative sera.

DISCUSSION

Different serological assays measure diverse subsets of MV antibodies, and it is not clear which subset reflects the cellular immune response best. Whole MV-based ELISAs detect a broad range of immunoglobulins, irrespective of their functional activities (8, 15, 35, 50). Functional assays, such as HI and NT, are therefore considered to provide a better estimate of the immune status. Hemagglutination-inhibition and neutralization antibodies are mainly H protein specific (21, 31). Our study demonstrates for a large cohort of human serum samples that antibody levels measured by an ELISA based on

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the recombinant H protein expressed in a mammalian system correlate considerably better with HI and NT titers than those measured by conventional whole-virus-based ELISAs, indicating that the H-ELISA measures predominantly functional antibodies. Since hemagglutination-inhibition and neutralization antibodies are strongly associated with in vivo protection (11, 21), our results also suggest that the H-ELISA may be more appropriate than MV-ELISAs for measuring immunity to measles.

Sera negative by both HI and NT and positive by both HI and NT served to find the thresholds for positivity (120 mOD units) and negativity (80 mOD units) of the H-ELISA. Since HI is normally less sensitive than NT (2, 34, 50) (Fig. 3), negativity by both HI and NT more rigorously defines negative individuals than negativity by NT alone (12, 34). Titration of the 2nd International Standard for anti-measles serum obtained from the National Institute for Biological Standards and Control (16) confirmed that the threshold for positivity given above corresponds to a protective antibody level: 120 mOD units corresponds to 215 mIU/ml (data not shown); different investigators (18, 33, 37) have considered concentrations of 200 (corresponding to 114 mOD units) to 255 mIU/ml to correspond to protective immunity. Under the assumption that the antibody specificities of test and standard sera are similar, this may suggest that individuals who are seropositive by the H-ELISA have protective immunity.

On the basis of the thresholds presented above and the results presented in Fig. 2, it is concluded that a positive serum sample has a chance of 209 in 212 (98.6%; with 1 serum sample being undefined) or 211 in 212 (99.5%) of testing positive and a negative serum has a chance of 10 in 11 (90.9%; with 1 negative serum sample being undefined) or 3 in 11 (27.3%; with 2 serum sample being undefined) of testing negative by H-ELISA or MV-ELISA, respectively. The assays did not dif-
fer significantly in the numbers of false-negative sera (for the H-ELISA, 2 of 212 [0.94%]; for the MV-ELISA, 1 of 212 [0.47%]). In contrast, significantly more serum samples had false-positive results by the MV-ELISA than by the H-ELISA (6 of 11 versus 0 of 11, respectively; \( P < 0.05 \)).

Although the number of negative serum samples was limited, the performance characteristics of the H-ELISA were determined (Table 1). The specificity, accuracy, and positive predictive value were generally better for the H-ELISA than for the MV-ELISA, independent of whether HI, NT, or HI and NT was used as the “gold standard.” In contrast, the H-ELISA proved to be slightly less sensitive than the MV-ELISA (98.6 versus 99.5% [99.6%], according to the supplier). The H-ELISA was also better by all parameters than HI (data not shown) (12, 34).

When undefined sera were included the MV-ELISA detected as negative only 5 serum samples among the 11 serum samples negative by both HI and NT. Although in larger seronegative cohorts between 58 and 100% of the serum samples were found to be negative by the same or different whole MV-based (fully optimized) commercial ELISAs (12, 34, 37), our data seem to suggest that the H-ELISA may be more efficient for identifying seronegative individuals.

Five of the six serum samples which were false positive by MV-ELISA were from 15 month-old, unvaccinated children. Discrepancies between ELISA and NT or HI measurements have been reported for those with maternally acquired antibodies (36, 37, 39). The results presented above indicate that these antibodies do not seem to interfere with our H-ELISA. Measurements of specific MV IgG antibodies should, most importantly, predict susceptibility to measles infection. In any given cohort, only a few individuals will be seronegative for measles. Among these, false-positive donors are at risk of disease and can support viral circulation. Identification of such false-positive sera would require the retesting of most serum samples (by a different assay). In contrast, individuals who test false negative have no enhanced risk and are epidemiologically irrelevant. Also, rare false-negative sera could, in principle, be retested (by another assay) or such individuals could simply be vaccinated or revaccinated. For these reasons, false-negative sera...
results can be better tolerated than false-positive results, which did not occur by our assay.

The global measles eradication efforts of the World Health Organization require an inexpensive and standardized source of antigen for surveillance of measles immunity, in particular, for the detection of seronegativity. Our data demonstrate that the H-protein preparation derived from the Semliki Forest virus expression system could be a simple alternative to current whole-virus-based ELISAs for surveillance of measles immunity and that such an assay could be more efficient in detecting susceptibility to measles. More importantly, however, unlike for antigens based on MV-infected cells, the properties of the H-protein antigen such as its stability are more compatible with the development of a simple field test for the diagnosis of measles, which is another requirement of eradication programs.

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REFERENCES


proteins of the attenuated measles virus strain AIK-C. Virus Res. 26:167–175.