Evaluation of Antibodies against a Rubella Virus Neutralizing Domain for Determination of Immune Status

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The protective immune responses against rubella virus (RV) are related to its neutralizing epitopes, an issue that is important to consider when assessing the immune status of patients with remote infection. In the present paper, we compare the antibodies detected by a synthetic-peptide-based enzyme immunoassay (EIA) with antibodies detected by the traditional technique of hemagglutination inhibition (HIA) in patients with remote RV infection. The synthetic peptide used as an antigen (SP15) represents a neutralizing epitope that corresponds to amino acids 208 to 239 of the E1 glycoprotein. The SP15-EIA was developed, all variables that affected the assay were standardized, and the test was validated using reference sera. Serum samples (n = 129) from patients with remote RV infection were tested by HIA and SP15-EIA. Discrepant sera were assayed by MEIA (IMX/Abbot). The comparison between HIA and SP15-EIA, taking HIA as the standard methodology for determining immune status, showed that SP15-EIA is very specific and sensitive for detecting protecting antibodies (specificity, 100%; sensitivity, 98.20%). This study demonstrates that antibodies against the neutralizing domain represented by SP15 would be important in the memory response after natural infection and may be a good tool in the determination of the true immune status of patients with remote infection with regard to RV.

Rubella virus (RV) is the etiologic agent of German measles and is the sole member of the genus Rubivirus in the Togaviridae family. During the first trimester of pregnancy, the infection may induce congenital malformations and viral persistence in the human fetus (26).

The RV virion contains an RNA genome enclosed in an icosahedral capsid composed of protein C (33 kDa). Surrounding this nucleocapsid is a lipid bilayer, in which viral glycoproteins E1 (58 kDa) and E2 (42 to 47 kDa) are embedded (18). The humoral immune response to RV is predominantly to the E1 glycoprotein and persists indefinitely after infection (13, 17).

The E1 glycoprotein has been suggested to be the immunodominant antigen, since most virus-neutralizing antibodies are directed against this subunit. Monoclonal antibodies (MAbs) were used to define the neutralizing domains on the E1 glycoprotein whose amino acid sequences were determined by overlapping synthetic peptides (9, 11, 12, 14, 21, 22, 24). One of these domains was defined by three independent MAbs that recognized the same sequence, represented by the synthetic peptide SP15 (E1 amino acids 208 to 239) (4, 25). Moreover, SP15 was shown to induce polyvalent antibodies with neutralizing and hemagglutination inhibition activity in mice and rabbits. The sequence of SP15 is present in several strains of RV, such as Therien, Judith, M33, HPV77, RA27/3, Gilchrist, wild-type Cordoba, and Kara 95 (5, 25).

Other authors using a similar synthetic peptide, BCH-178C (E1 amino acids 213 to 239), showed the existence of human antibodies that recognize this domain (15, 16, 27). These authors indicate that BCH-178C can favorably replace current viral lysate antigens for detection of RV immunoglobulin G antibodies following rubella vaccination. The increase of antibodies to this domain was also proved after vaccination of seronegative and seropositive individuals.

The hemagglutination inhibition assay (HIA) and neutralization assay are used for detecting protecting antibodies to RV. Oshea et al. (19) demonstrated that neutralizing antibodies detected by neutralization assays may not be useful in protecting from reinfection. Moreover, they concluded that protection must be associated with immune responses specific for the protective epitopes of rubella virus, an issue that is important to consider when measuring the immune status of patients with remote infection.

In the present paper, we compare an enzyme immunoassay (EIA) based on the use of SP15 as an antigen with the traditional technique of HIA for detecting protecting antibodies in patients with remote RV infection. Although it is well known that HIA is highly specific but not very sensitive compared with EIAs, at the moment it is considered the “gold standard” method for the determination of protective immunity to RV; that is the reason we used HIA to validate our SP15-EIA.

MATERIALS AND METHODS

Clinical specimens. A total of 121 human serum samples were tested. Samples were taken from women (20 to 35 years old) without a recent history of exanthematous illnesses or contact with rubella patients.

HIA. The HIA was described previously by Palmer et al. (20) and Cordoba et al. (3). The hemagglutinating antigen was obtained by alkaline extraction from RV-infected Vero cells (20).

SP15-EIA. SP15 peptide was kindly provided by Jerry Wollinsky (University of Texas, Houston). SP15 was synthesized by the solid-phase method based on the standard tert-butylxycarbonyl amino acid addition protocol. The assay was performed as follows: 100 μl of the synthetic peptide SP15 (40 μg/ml) diluted in sodium carbonate buffer (pH 9.6) was attached to PoliSorp Nunc-Immuno modules and kept overnight at room temperature. After washing with phosphate-buffered saline (PBS)-TWEEN 20, the wells were blocked with 3% bovine serum albumin–1% calf serum in PBS for 2 h at 37°C. The modules were washed three times with PBS-TWEEN 20 and incubated with human sera (diluted 1:50 in PBS) for 1 h at 37°C. After another washing step, horseradish peroxidase-conjugated goat anti-human immunoglobulin (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) diluted in PBS was added to each well and incubated for 1 h at 37°C. The modules were newly washed with PBS-TWEEN 20 and developed by addition of tetramethylbenzidine-H2O2 (TMB peroxidase EIA substrate kit;
Bio-Rad Laboratories, Hercules, Calif.). The reaction was stopped by addition of 2 N H₂SO₄, and the optical density at 450 nm was read.

A panel of 16 human sera assayed by HIA with results confirmed by MEIA (Abbot/IMX) was used for the standardization of SP15-EIA, which comprised eight negative, four high-positive, and four low-positive sera.

Five negative, one high-positive, and one low-positive serum sample were used as controls in each assay. The cutoff value was obtained from the read of the negative controls. SP15-EIA results were expressed as an index with respect to the cutoff value. When the index was higher than 1, the sample was considered positive, and it was considered negative when the index was lower. The calculations and determination of test validity were made separately for each assay using the following steps and criteria. Control wells without antigen must be <0.005. At least three of the five negative controls must be <0.250. The mean value of negative controls and the standard deviation are determined. The low-positive control value minus the mean value of negative controls must be >0.14. If the test is valid, the cutoff value is obtained as the mean value of negative controls plus 2 standard deviations. The index value is obtained as the optical density of the sample divided by the cutoff value.

RESULTS

One hundred twenty-one serum samples obtained from patients with remote RV infection were tested using HIA and SP15-EIA. In these tests, 98 samples were positive by both HIA and SP15-EIA, 4 were positive by HIA but negative by SP15-EIA, 6 were negative by HIA and positive by SP15-EIA, and 13 were negative by both assays. These results indicated a specificity for SP15-EIA of 68.40% and a sensitivity of 96.07%.

The discrepant sera (six that were EIA positive and HIA negative and four that were EIA negative and HIA positive) were tested by MEIA (IMX/Abbot). All six sera initially labeled as EIA false positive were confirmed as positive samples. Two of the four EIA false-negative sera were positive by MEIA, and the other two were undetermined. In all, considering the results of the analysis of discrepant sera by MEIA, 106 samples were positive by SP15-EIA and HIA, 2 were positive by HIA but negative by SP15-EIA, 13 were negative by both assays, and none were negative by HIA but positive by SP15-EIA, giving a specificity of 100% and sensitivity of 98.15% for SP15-EIA.

DISCUSSION

At the moment, Argentina lacks a program of massive vaccination against RV, and cases of congenital rubella syndrome (CRS) are still occurring. In other countries, where all school-age children are vaccinated, CRS cases are arising due to reinfection of pregnant women (1, 2, 6). Thus, the determination of protective immunity to RV plays a key role in deciding whether the vaccination of a susceptible woman is indicated and in further testing the effectiveness of the vaccination.

The immune response to RV infection induces antibodies with specificities for different epitopes. All these antibodies can be measured by available immunoassays that detect the formation of immune complexes, whereas the neutralization assay and HIA reflect a specific biologic function of the antigen. Although neutralizing antibodies can prevent transmission of virus to the fetus following rubella reinfection, Oshea et al. (19) showed that rubella reinfection is not always associated with the lack of neutralizing antibodies. HIA, which is considered the gold standard for immune status determination (7), detects antibodies against neutralizing and/or hemagglutinating epitopes with a low sensitivity. In this way, commercial EIAs may not be strictly comparable with neutralization assays or HIA in detecting all antibodies induced by the infection, since the mere presence of antibodies does not ensure protection from reinfection. Conversely, negative results by HIA do not always constitute an argument for revaccination. Are pre-existing antibodies used as a measure of immune status really neutralizing?

We developed an SP15-EIA that was compared with the traditional HIA for the determination of immune status for 121 patients with remote natural infection. This allowed us to relate antibodies against a neutralizing epitope to antibodies against the viral hemagglutinin. All variables that affected the assay were standardized, and the test was validated using sera previously assayed by two other methods, one of them considered the gold standard (HIA) and the other a very sensitive one (MEIA/IMX) that also detects SP15-directed antibodies (data not shown).

When HIA was used as the standard methodology for determining immune status, the SP15-EIA was very specific and sensitive for detecting protective antibodies. The SP15-EIA false-positive sera were assayed by MEIA (Abbot/IMX) and confirmed as positive. Mitchell et al. (16) found low levels of immunoglobulin G antibodies to the BCH-178C synthetic peptide in military individuals who had been vaccinated in their infancy. Both results suggest that preexisting neutralizing antibodies against this epitope could be more important as a memory response after natural infection than after vaccination and could explain why reinfection occurs more frequently in vaccinated than in naturally immune individuals.

HIA is the standard methodology for determining immune status. The results presented in this report indicate that SP15-EIA has a better capacity to detect true negatives. As a result, vaccination is recommended for patients without antibodies, but protected individuals are not unnecessarily vaccinated. This raises the question of whether HIA is a true gold standard method for determining immune status, suggesting that HIA could be replaced by a more sensitive technique based on synthetic peptides that represent neutralizing epitopes. Synthetic peptides have proven to be useful in viral immunodiagnosis (8, 10, 23, 27). This study indicates that SP15 may be an alternative antigen for use in EIAs and suggests that SP15-EIA is a sensitive and specific method for the determination of protecting antibodies against RV.

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