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

PATRICIA CORDOBA, ALEJANDRA LANOEL, SERGIO GRUTADAURIA, AND MARTA ZAPATA
Instituto de Virologia, Facultad de Ciencias Medicas, Universidad Nacional de Cordoba, Cordoba, Argentina

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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 following rubella vaccination. The increase of anti-viral lysate antigens for detection of RV immunoglobulin G thors indicate that BCH-178C can favorably replace current antibodies that recognize this domain (15, 16, 27). These au-


domains was defined by three independent MAbs that were used to define 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 protective 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 exanthematic 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;
existing antibodies used as a measure of immune status really not always constitute an argument for revaccination. Are pre-
erved the gold standard for immune status determination (7), (19) showed that rubella reinfection is not always associated
virus to the fetus following rubella reinfection, Oshea et al. Although neutralizing antibodies can prevent transmission of
and HIA reflect a specific biologic function of the antigen. formation of immune complexes, whereas the neutralization assay
be measured by available immunoassays that detect the for-
tion of protective immunity to RV plays a key role in deciding
age children are vaccinated, CRS cases are arising due to
resulting in military individuals who had been vaccinated in their
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. Syn-	hetic 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.

RESULTS
One hundred twenty-one serum samples obtained from pa-
tients 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 neg-
ative and four that were EIA negative and HIA positive) were
tested by MEIA (IMX/Abbott). 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 re-

tults 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 vac-
cination 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 determina-
tion 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 for-
mation 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 consid-
cered the gold standard for immune status determination (7),
detects antibodies against neutralizing and/or hemagglutinat-
ing 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 protec-
tion 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 consid-
ered 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 de-
termining immune status, the SP15-EIA was very specific and
relevant for detecting protecting antibodies. The SP15-EIA
false-positive sera were assayed by MEIA (Abbott/IMX) and
confirmed as positive. Mitchell et al. (16) found low levels of
immunoglobulin G antibodies to the BCH-178C synthetic pept-
ide in military individuals who had been vaccinated in their
infancy. Both results suggest that preexisting neutralizing an-
tibodies 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.

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but protected individuals are not unnecessarily vaccinated. This raises the question of whether HIA is a true gold standard
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