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Serological Detection of Varicella-Zoster Virus-Specific Immunoglobulin G by an Enzyme-Linked Immunosorbent Assay Using Glycoprotein Antigen

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Since the introduction of varicella vaccination in several countries, there has been an urgent need for commercially available test procedures that allow highly sensitive and specific quantitative determination of the varicella-zoster virus (VZV)-specific immune status, including immunity postimmunization. This study compared the performance of two enzyme-linked immunosorbent assays (ELISAs) for the sensitive and specific determination of VZV-specific immunoglobulin G (IgG) in seronegative and latently infected persons, as well as in vaccinees. One ELISA is based on the detection of antibody to VZV-specific envelope glycoproteins (gp), and the other comprises the whole antigen extract prepared from VZV-infected cells. A modified standard fluorescent-antibody-to-membrane-antigen (FAMA) assay was used as a reference. An excellent sensitivity (100%) in relation to FAMA was demonstrated for the gpELISA (Virion\Serion), while the non-gpELISA (Dade Behring) had a lower sensitivity (83%) when sera from latently infected persons were tested. After postvaccinal immunity was measured, a sensitivity of 87% was achieved with gpELISA, whereas the ELISA incorporating antigen extract of VZV-infected cells had a sensitivity of 78%. Excellent specificity (100%) was calculated for both the gpELISA and the non-gpELISA. In conclusion, SERION ELISA *classic* VZV IgG is useful for the sensitive and specific quantitative determination of VZV immune status after natural infection. The test can also be recommended for measuring antibody response after varicella vaccination, particularly after the cutoff value was optimized.

The determination of specific immunoglobulin G (IgG) is of great significance for obtaining serological proof of immunity to varicella-zoster virus (VZV) since patient histories cannot be regarded as reliable indicators of past primary infections (9). In particular, laboratory determination of the status of immunity to VZV has been recommended (i) in immunocompromised patients after exposure to VZV and prior to varicella vaccination (11), (ii) in pregnant women or those considering pregnancy with a history of exposure to VZV or an uncertain history with regard to varicella (19), and (iii) in health workers prior to varicella vaccination or after exposure to VZV (3). After the introduction of universal varicella vaccination in a number of countries, such as in Germany (14), the indications for measuring VZV-specific IgG antibodies have been broadened. In particular, a postimmunization serological testing of immunity has been required for immunocompromised vaccinees and health workers (18). In addition, quantitative monitoring of changes in VZV antibody levels in a population is a component of varicella vaccination surveillance (10).

In most diagnostic laboratories, the methodological opportunities for testing immunity after varicella vaccination are fairly limited. The main reason for that is the low sensitivity of the currently available commercial laboratory assays, in particular of different modifications of the enzyme-linked immunosorbent assay (ELISA) (6). A general consensus is that the most reliable assay for determining the status of immunity to VZV is the fluorescent-antibody-to-membrane-antigen (FAMA) test detecting antibodies specific to the viral envelope glycoproteins (gp) (4, 7). Thus, these antibodies can reflect the specific cellular immunity that plays the key role in protection against VZV infections (5). However, the time-consuming procedure for measuring cellular immunity precludes its routine use (22). To date, the FAMA procedure can only be performed as a modified in-house test that is labor-intensive and requires considerable experience in handling VZV, and interpretation of the test results is subjective. Since there is no sufficient experience in storage of FAMA, the results can only be obtained within several days. Furthermore, the use of diploid fibroblasts derived from different human origins generally prevents attempts to achieve standardization and automation. Practical experience with the FAMA kit commercially produced by Viran Clinical Diagnostics (Stevensville, MI) suggested that the results are not reproducible (1). Finally, Merck Research Laboratories (West Point, PA) developed VZV gp-specific ELISA (21), but this assay is not available commercially. In conclusion, there is an urgent need for commercially distributed test procedures that allow a highly sensitive and specific quantitative determination of VZV immune status, including immunity after varicella vaccination.

In the present study, the SERION ELISA *classic* VZV IgG, produced by the Institut Virion\Serion, Würzburg, Germany, was evaluated for its sensitivity and specificity. The viral antigen of this test contains VZV-specific envelope gp. The SERION ELISA was compared to the ELISA Enzygnost Anti-VZV/IgG distributed by Dade Behring, Marburg, Germany. This ELISA, which has been used in most diagnostic laboratories, is based on the whole-antigen extract prepared from

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VZV-infected cells. Defined panels of sera from seronegative and latently infected persons, as well as from vaccinees after varicella immunization, served as probes. A modified standard FAMA assay was used as the reference procedure.

MATERIALS AND METHODS

Serum panels. The sera used for the present study were obtained from voluntary blood donors and varicella vaccinees. Patient consent was obtained prior to processing the samples. The sera were tested utilizing a reference procedure for the determination of VZV-specific IgG class antibodies and stored in aliquots at −20°C without interruption. According to the test results, the sera were classified into the following four panels: (i) 25 serum samples from VZV-seronegative persons that were FAMA/anti-VZV IgG negative and indirect fluorescence antibody test [IFAT]–anti-herpes simplex virus [HSV] IgG negative; (ii) 25 serum samples from VZV-seronegative persons that were FAMA/anti-VZV IgG negative and IFAT–anti-HSV IgG positive; (iii) 50 serum samples from persons who were latently infected with VZV that were FAMA–anti-VZV IgG weakly or moderately positive in titers of 1:2 to 1:16 (mean titer, 1:5); and (iv) 80 serum samples from persons who were vaccinated against varicella by using Varilrix (GlaxoSmithKline, Uxbridge, United Kingdom) that were FAMA–anti-VZV IgG weakly, moderately, or highly positive in titers of 1:4 to 1:128 (mean titer of 1:19). Immediately before vaccination, the vaccinees were determined by testing to be VZV seronegative. Sera were obtained ≥6 weeks after vaccination using one dose of the Varilrix vaccine.

Serum panel ii was used to exclude cross-reactions of VZV antigens to HSV-specific antibodies. All sera were allowed to achieve room temperature immediately before investigation. IgG class antibody testing was carried out blindly in groups of 20 to 30 serum samples.

Reference procedure. The FAMA test, which has been considered the "gold standard" for determining the status of immunity to VZV, was used as reference procedure for the evaluation of SERION ELISA *classic* VZV IgG compared to the Enzygnost Anti-VZV/IgG test. FAMA has frequently been used as a reference for biologically relevant anti-VZV antibodies specific to the gp of the viral surface (7). The test was performed as an in-house modification of the standard version (24) described recently (17, 23). In short, suspensions of human embryonal lung fibroblasts seeded into each well of flat-bottom microtiter plates were infected with the VZV Oka strain at a multiplicity of infection of about 0.005. The medium used was Eagle minimal essential medium with Earle's balanced salt solution and 25 mM HEPES (Cambrex BioScience, Verviers, Belgium) supplemented with 1% (vol/vol) L-glutamine, 100 U of penicillin/ml, 100 μg of streptomycin sulfate/ml, and 10% fetal calf serum. After cytopathic effects were observed in ca. 30 to 50% of the cells, the cell monolayers were fixed with 0.1% glutaraldehyde (24) at 4°C for 1 min and washed three times with phosphate-buffered saline. Thereafter, the VZV-infected cells were incubated in the microplate cavities with the serially diluted sera at 37°C for 30 min and washed with phosphate-buffered saline. The cell preparations were covered with fluorescein isothiocyanate-labeled anti-human IgG from rabbit (Dako, Hamburg, Germany), mixed with Evans blue, and subsequently washed again. Finally, the infected cells were evaluated by using the inverse fluorescence microscope DIAPHOT-TMD (Nikon, Tokyo, Japan). Titers of ≥1:2 were considered positive.

An in-house modification of the IFAT was used for the determination of HSV-1/2-specific IgG levels. HSV-2 US strain-infected HEp-2 cells grown on glass microscope slides served as antigens (16).

ELISAs to be tested. The test SERION ELISA *classic* VZV IgG (no. ESR 104 G, lot SFV.DD; Institut Virion\Serion, Würzburg, Germany) evaluated in the present study is based on the determination of specific IgG class antibodies to viral envelope gp of VZV. The VZV strain Ellen (American Type Culture Collection VR 1367) grown in human embryonal lung fibroblasts was used for the production of viral antigens bound to the surface of microtitration wells. VZV gp antigen was prepared according to a modified method described previously (21) and purified by means of affinity chromatography using a lentil lectin-Sepharose 4B column (Pharmacia Biotech, Uppsala, Sweden). For testing sera, the manufacturer's instructions were followed. Test samples were diluted 1 in 100 in dilution buffer. Plates were washed by using the microplate washer aw1 (Anthos Labtec Instruments, Wals, Austria) and read photometrically using an Anthos reader 2010 (Anthos Labtec Instruments) at 405 nm and a reference wavelength of 620 nm. VZV antibody levels were expressed in mIU/ml, which were calculated by one-point calibration of a standard curve by using the software Serion Evaluate (version 2.21; Institut Virion\Serion). According to the recommendation of the Robert Koch-Institute (RKI) (13), positive results were equivalent to >100 mIU of anti-VZV IgG/ml, equivocal results were equivalent to 50 to 100 mIU/ml, and results of <50 mIU/ml were considered negative.

Results of SERION ELISA *classic* VZV IgG were compared to that of the commercial ELISA Enzygnost Anti-VZV/IgG (no. OWLT15, lot 34609; Dade Behring, Marburg, Germany) since this test has been most commonly used for routine diagnostic determination of VZV immune status. In contrast to the SERION ELISA, the microtitration plates of the Dade Behring ELISA were coated with inactivated viral antigen, which represents an extract from VZV-infected cells and does not contain concentrated and purified VZV-specific gp. The Enzygnost ELISA was performed according to the manufacturer's instruction. The anti-VZV reference serum and the test samples were prediluted 1 in 21 using sample buffer and, afterward, the resulting predilution was diluted 1 in 11 before starting incubation. Plates were washed by using the microplate washer aw1 (Anthos Labtec Instruments). Extinction at 405 nm and the recommended reference wavelength of 620 nm was measured by using the Anthos reader 2010 (Anthos Labtec Instruments). Quantitative results were calculated with the aid of the α-method using the Behring's pocket calculator. Antibody levels expressed in mIU/ml, based on the WHO international standard for varicella-zoster immunoglobulin (50 IU), were assessed in the same manner as described for the SERION ELISA *classic*.

Statistical methods. Sensitivity was calculated as the proportion of positive sera (VZV IgG positive using the reference test) that were correctly identified as positive by the ELISAs SERION ELISA *classic* VZV IgG or Enzygnost Anti-VZV/IgG. Specificity referred to the proportion of negative sera (VZV IgG negative using the reference test), which had a negative test result using the SERION ELISA *classic* VZV IgG or ELISAs Enzygnost Anti-VZV/IgG. Correlation was computed for the SERION ELISA *classic* VZV IgG or the Enzygnost Anti-VZV/IgG ELISAs as the number of sera with concordant results divided by the number of sera included. Equivocal results were excluded before the statistical calculation was carried out.

RESULTS

The results of all four serum panels tested are summarized in the Table 1. As shown, there was an absolute concordance between the negative results of the SERION ELISA *classic* VZV IgG and the Enzygnost Anti-VZV/IgG and the reference procedure concerning serum panel 1 ($n = 25$; VZV-specific IgG antibody negative, HSV IgG antibody negative) and serum panel 2 ($n = 25$; VZV-specific IgG antibody negative, HSV IgG antibody positive), apart from one equivocal result using the SERION ELISA *classic* in serum panel 2. Testing of panel 3, which consisted of 50 sera from latently infected persons with weakly or moderately positive VZV IgG levels, revealed 46 positive and 4 equivocal results in the SERION ELISA *classic*. In contrast, there were 39 positive, 8 negative, and 3 equivocal results in this serum panel using the Enzygnost ELISA. Discrepant results were most frequently found in the sera of panel 4 containing 80 samples derived from persons ≥6 weeks after varicella vaccination. Whereas the FAMA as a reference procedure revealed weakly, moderately, or highly positive titers, SERION ELISA *classic* was positive in 53, negative in 8, and equivocal in 19 sera. Using Enzygnost, 50 of the sera were determined to be positive, in 14 sera VZV-specific antibodies could not be detected, and 16 sera yielded an equivocal result. In summary, there were 24 equivocal results using the SERION ELISA *classic*, 23 of which were positive and 1 of which was negative in the reference method. The Enzygnost ELISA revealed 19 equivocal results, all of which were positive in the reference test.

After exclusion of all equivocal results, 100% specificity was calculated for SERION ELISA *classic* VZV IgG as well as the ELISA Enzygnost Anti-VZV/IgG (Table 2). For the SERION ELISA, the sensitivity for detection of natural VZV immunity in latently infected persons was 100% and for detection of

TABLE 1. Results achieved by SERION ELISA *classic* VZV IgG in comparison with Enzygnost Anti-VZV/IgG

Serum panel (*n*)[a]	No. of samples					
	SERION ELISA Classic VZV IgG			Enzygnost Anti-VZV/IgG		
	Positive	Equivocal	Negative	Positive	Equivocal	Negative
VZV IgG negative (25)	0	0	**25**	0	0	**25**
VZV IgG negative, HSV-1/2 IgG positive (25)	0	1	**24**	0	0	**25**
VZV IgG weakly or moderately positive (50)	**46**	4	0	**39**	3	8
After varicella vaccination (80)	**53**	19	8	**50**	16	14

[a] VZV IgG levels were determined using FAMA as a reference procedure. Values representing the results of the referrence procedure are indicated in boldface. *n*, number of serum samples.

postvaccinal immunity a sensitivity of 86.9% could be calculated (overall sensitivity 92.5%). In comparison, Dade Behring ELISA Enzygnost had a sensitivity of 83.0% to determine natural immunity and a sensitivity of 78.1% for determination of immunity post varicella vaccination (overall sensitivity, 80.2%). There was 94.9% correlation between the results of SERION ELISA *classic* and the FAMA as reference procedure when equivocal results were excluded. Using Enzygnost Anti-VZV/IgG, a lower correlation of 86.3% was calculated.

DISCUSSION

Several studies to evaluate commercial or in-house tests for measuring VZV-specific IgG antibody such as ELISAs, immunofluorescence assays, and latex agglutination assays have been reported. Most of these investigations assessed humoral immunity following natural primary infection. To date, only few studies have examined VZV IgG in response to varicella vaccination. However, these studies all correlated in demonstrating a reduced sensitivity compared to FAMA when postvaccinal immunity was assessed (1, 2, 15, 20). The use of a whole-antigen extract from VZV-infected cells containing a variety of VZV structural and nonstructural proteins has been shown as the most likely reason for that since a significantly greater sensitivity of ELISAs using purified viral gp antigen has been demonstrated (12, 21). Thus, the gpELISA developed by the Merck Research Laboratories in the United States has a sensitivity comparable to that of FAMA, and the results agree with neutralizing antibodies (8). Unfortunately, this test is restricted to a small number of research laboratories and is not commercially available to date.

In the present study, we evaluated SERION ELISA *classic* VZV IgG, which is to the our knowledge the first commercially available gpELISA for the detection of VZV IgG antibodies. The test has been distributed by the Institute Virion\Serion in Germany. As samples, different serum panels derived from seronegative individuals, latently infected persons, and vaccinees were assembled. The results were compared to those of the FAMA as a reference procedure and with those of the Dade Behring ELISA Enzygnost Anti-VZV/IgG, which is most commonly used in diagnostic laboratories in Germany. The results were quantitatively assessed on the basis of the RKI recommendations (13), which were identical with the manufacturers' instructions. This means that positive results were >100 mIU/ml, equivocal results were 50 to 100 mIU/ml, and results of <50 mIU/ml were considered negative. However, it should be considered that these recommendations are only applicable to the determination of VZV immune status in nonvaccinated persons. For the SERION ELISA *classic*, a specificity of 100% and overall sensitivity of ca. 93% was calculated. There was an excellent sensitivity of 100% for determining VZV immunity in latently infected persons, and the sensitivity for measuring immunity in response to vaccination was slightly reduced (86.9%). These results seem to be surprising at first glance since the mean titer of FAMA in sera from latently infected persons was considerably lower (mean titer of 1:5) than the mean titer in the group of vaccinees (mean titer of 1:19). However, these findings reflect the determination of gp-specific antibody to VZV using FAMA and its measurement by the SERION ELISA to a lower extent. A disadvantage is that an internationally accepted standardized reference serum containing defined amount of VZV gp antibody is not available for the direct comparison of the methods to date.

TABLE 2. Sensitivity, specificity, and correlation of SERION ELISA *classic* VZV IgG and Enzygnost Anti VZV/IgG compared to FAMA as a reference procedure

ELISA tested	Specificity (%)	Sensitivity (%)[a]	Correlation (%)
SERION ELISA *classic* VZV IgG	100	100/86.9	94.9
Enzygnost Anti-VZV/IgG	100	83.0/78.1	86.3

[a] Expressed as natural immunity/postvaccinal immunity.

Due to the relatively high number of sera with equivocal results achieved by the SERION ELISA, further optimizing the cutoff value would improve the sensitivity of this test, particularly in patients whose postvaccinal immunity requires assessment. For example, if the cutoff value was reduced to 50 mIU/ml, all equivocal results could be interpreted as positive without any significant loss of test specificity. In this case, the SERION ELISA would be characterized by a specificity of 98% and a sensitivity of 90% in vaccinees. This suggests that the results are comparable to those of FAMA when immunity after varicella vaccination is measured. Thus, the evaluated SERION ELISA *classic* could be recommended not only for the sensitive and specific quantitative determination of VZV immune status in naturally infected persons but also for measuring the vaccine response after the cutoff value is optimized. The small number of negative sera might be retested using FAMA or the immunofluorescence test recently described as an alternative to FAMA (17).

The Enzygnost ELISA from Dade Behring, whose performance was compared to that of the SERION ELISA *classic*, had a reduced overall sensitivity of 80.2% and an excellent

specificity of 100% in relation to FAMA. Sensitivity was improved when sera from latently infected persons were tested (83%) in comparison to the sera of vaccinees (8.1%). These findings are in contrast to the results recently published by Maple et al. (10). After the VZV immune status in an adult population was measured, a very good sensitivity of 98.4% and a diminished specificity of 80.7% have been reported for the Dade Behring ELISA distributed in United Kingdom. These results were in relation to a time-resolved fluorescence immunoassay that had high sensitivity and specificity compared to the gpELISA from Merck. Provided that there are no methodological differences between the ELISAs distributed in the United Kingdom and Germany, these contradictory results in terms of sensitivity and specificity are difficult to explain. However, the use of distinct study groups and reference procedures may generally lead to different results in both studies. Compared to the gpELISA, which contains a more concentrated and purified viral antigen, a loss of sensitivity as well as specificity can be expected for ELISAs using the whole antigen extract of VZV-infected cells such as in the Dade Behring procedure. However, according to the manufacturer, the Dade Behring ELISA can be used to check whether a VZV vaccination has been successful, our results indicate no sufficient sensitivity for this purpose. This is also correct if the equivocal results are considered positive.

In summary, because of the good correlation with the FAMA assay, the SERION ELISA *classic* VZV IgG is useful for sensitive and specific quantitative determination of VZV-specific immune status following natural infection. The test can also be recommended for measuring antibody response after varicella vaccination, particularly after optimizing the cutoff value.

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