ELSEVIER Journal of Virological Methods 53 (1995) 176–187

Journal of Virological Methods

Combined use of complement and anti-immunoglobulin in an enhanced neutralization assay for antibodies to varicella-zoster virus

David. L. Krah *, Philip J. Provost, Ronald W. Ellis

Department of Virus and Cell Biology, Merck Research Laboratories, West Point, PA 19486, USA

Accepted 30 January 1995

Abstract

An enhanced neutralization assay was developed to permit the sensitive, specific, and reproducible measurement of antibodies to varicella-zoster virus (VZV). Optimal neutralization was achieved using a combination of guinea pig complement (C′) and rabbit anti-human IgG. This provided 625-, 160- and 13- to 64-fold increases in dilution endpoints of human post-zoster serum, varicella-zoster immune globulin and representative sera from recipients of live attenuated varicella vaccine, respectively, above those measured in the absence of C′ and anti-IgG. The specificity of the assay was shown by the absorption of serum neutralization capacity with VZV-specific antigen and the lack of concordance between antibody titers to VZV with those to either herpes simplex virus type-2 or cytomegalovirus. The antibody status of recipients of live attenuated varicella vaccine was established from the amount of neutralizing activity produced at a single optimal serum dilution (1 : 64).

Keywords: Varicella-zoster virus vaccine; Enhanced neutralization assay; Vaccine-induced antibody; Antiviral antibody

1. Introduction

Infection with varicella-zoster virus (VZV), the causative agent of chickenpox and shingles, elicits both humoral and cell-mediated immune responses. Antibody assays are used to identify prior infection and to predict susceptibility to disease and have included

* Corresponding author. Fax: +1 (215) 652 7320.

SSDI 0166-0934(95)00013-5

complement-fixation (Forghani et al., 1978; Cremer et al., 1985), immune adherence hemagglutination (Forghani et al., 1978), passive hemagglutination (Kino and Minamishima, 1993), latex agglutination (Steinberg and Gershon, 1991; Landry and Ferguson, 1993), fluorescent antibody to membrane antigen (Williams et al., 1974), enzyme-linked immunosorbent assays (ELISAs) (Shehab and Brunell, 1983; Wasmuth and Miller, 1990), and neutralization (Nt) (Caunt and Shaw, 1969; Schmidt and Lennette, 1975; Asano et al., 1982) assays. Virus Nt assays measure biologically active antibodies and therefore may offer a good surrogate of protection (Asano and Takahashi, 1978; Cremer et al., 1985).

Complement (C′) (Schmidt and Lennette, 1975) or anti-immunoglobulin antibodies (Asano et al., 1982, 1983) have been used individually to increase the sensitivity of VZV Nt assays. The combined use of C′ and anti-human IgG is described to sensitively measure VZV Nt antibodies. Optimal assay conditions and demonstration of the specificity of the Nt are presented. The utility is demonstrated of a qualitative assay, in which Nt activity is measured at a single optimal serum dilution to establish the serum antibody status of recipients of live attenuated varicella vaccine (Takahashi et al., 1975; White et al., 1991).

2. Materials and methods

2.1. Cells and virus

MRC-5 cells (fetal human diploid lung) and live varicella vaccine (Oka/Merck, Varivax®) were obtained and grown as described elsewhere (Krah et al., 1990). Two additional VZV preparations, cultured from vesicle scrapings of children with chickenpox, were also used in Nt experiments. These were identified as 'wild-type' virus from their restriction endonuclease fingerprints (Gelb et al., 1987). After development of cytopathic effect in at least 30% of the culture, cells were washed with phosphate-buffered saline (PBS), scraped into ice-cold PGS solution (PGS: PBS, sucrose, hydrolyzed gelatin, sodium glutamate) (M. Takahashi, personal communication), disrupted by sonication (15 s at maximal power using a 1/8-in. diameter microtip fitted on a model W140 sonicator (Heat Systems-Ultrasonics, Inc., Plainview, NY), and clarified by centrifugation at 325 *g* for 10 min. Antigens for use in serum absorptions were prepared from uninfected control or VZV-infected MRC-5 cultures. Cell-free VZV preparations were stored in single-use aliquots at −70°C.

2.2. Sera

Paired sera were kindly provided by C.J. White (Merck Research Laboratories) and were obtained from healthy children and adolescents (age range, 12 months to 15 years; median, 3 years) participating with informed consent in clinical trials of live varicella vaccine. This panel contained primarily weak responders (median titer < 2.5) or non-responders to the vaccine, as measured in the VARELISA assay (Shehab and

Brunell, 1983) for anti-VZV antibodies. The VARELISA utilizes total VZV-infected cell protein as the solid-phase antigen. Additional sera included a human post-zoster convalescent serum (HPZ), varicella zoster immune globulin (human VZIG, 125 U/tube, lot MVZIG-27, Massachusetts, Public Health Laboratories, Boston, MA), and a negative serum prepared from a pool of prevaccination human sera that registered as negative in the VARELISA and gpELISA (Wasmuth and Miller, 1990; Provost et al., 1991) assays for anti-VZV antibodies. The gpELISA assay employs lentil-lectin purified infected cell protein as the solid-phase antigen. Unless otherwise stated, sera were heat-inactivated at 56°C for 30 min before use in the Nt assay.

Pre- and postvaccination sera from two volunteers who received live cytomegalovirus (CMV) vaccine (serum pairs 896/953 and 970/977) were kindly provided by S. Plotkin (Children's Hospital of Philadelphia). Both individuals had seroconverted for anti-CMV antibodies in an anti-C′ immunofluorescence assay (ACIF) (S. Plotkin, personal communication).

Paired sera from 4 individuals before and after clinical flare-up of herpes simplex virus type-2 (HSV-2) infection (serum pairs 3/4, 11/12, 19/20, and 43/44) were kindly provided by R. Whitley (University of Alabama, Birmingham). Increases in specific anti-HSV-2 antibodies were demonstrated by immunoblot assay in all 4 postsera (S. Chatterjee, personal communication).

2.3. Neutralization assay

Sera were diluted in PGS solution either at a 1:64 dilution or at serial two-fold dilutions for determination of dilution endpoints. Controls included serial 5-fold dilutions (1:250 through 1:156,250) of HPZ serum, a negative control serum (1:64 dilution), and PGS alone ('no serum' control). Lyophilized live varicella vaccine (21,000 plaque-forming units (PFU)/ml) was reconstituted at 0–8°C with 0.7 ml sterile distilled water and diluted 1:15 (~ 140 PFU/100 μl) into PGS containing 74 50%-hemolytic (HLU)/ml of guinea pig C′ (or PGS alone), and 100-μl volumes were mixed with 200 μl of diluted serum in 2-ml capacity round-bottom polypropylene tubes (Corning, NY).

Following incubation at 35°C for 1 h, tubes received 100 μl of undiluted rabbit anti-human IgG (Cappel/Organon Teknica, West Chester, PA) or 100 μl PBS as control and were incubated for 30 min at 20–23°C, with gentle shaking at 10-min intervals. Remaining VZV PFU in triplicate 100-μl volumes of the Nt mixtures were quantified by plaque assay (Krah et al., 1990) using an overlay medium consisting of minimal essential medium with Earle's salts (MEM, GIBCO, Grand Island, NY), 2% heat-inactivated fetal calf serum (FCS), 50 μg/ml neomycin sulfate, and 2 mM L-glutamine.

Plaque counts from replicate plates were averaged. Nt was measured by two methods. In method 1, individual sera were judged Nt^+ if they produced a $\geqslant 50\%$ reduction in PFU relative to the 'no serum' control. In method 2, post-vaccination were judged Nt^+ if they produced $\geqslant 50\%$ Nt relative to the corresponding prevaccination serum. The endpoint of serial serum dilutions was defined as the highest dilution providing $\geqslant 50\%$ Nt.

2.4. Serum absorptions

Cell-free VZV and control preparations were heated at 56°C for 1 h to inactivate live virus and were stored at −70°C until used for absorptions. Sera (1 : 64 final dilutions of sera from vaccinees or 1 : 6250 dilution of HPZ serum) were incubated with PGS or PGS containing heat-inactivated VZV-infected MRC-5 cell extract (2.4 μg VZV antigen) or an equivalent amount of extract from uninfected MRC-5 cells in a total volume of 500 μl for 24 h at 4°C and then were assayed for Nt activity. VZV antigen was quantified by a competitive ELISA (D. Krah, unpublished data). Nt activity was measured by method 2 (for paired sera from vaccinees) or method 1 (HPZ serum).

2.5. Complement

Lyophilized guinea pig serum (Sigma Chemical Co., St. Louis, MO) (complement, C′) was reconstituted immediately before use in sterile distilled water held at 0–4°C, diluted with PGS, and sterile filtered through a 0.22-μm Millex-GV low protein binding filter (Millipore, Bedford, MA). Diluted C′ was held at 0–4°C for a maximum of 15 min. Hemolytic activity was determined by the manufacturer.

2.6. Secondary antibody preparations

The IgG fractions of rabbit anti-human IgG (heavy (H) and light (L) chain-specific, 5 mg/ml specific IgG and 20.7 mg protein/ml in PBS), goat anti-human IgG (H and L chain, 10 mg IgG/ml and 27.8 mg protein/ml), sheep anti-human IgG (H and L chain, 10 mg IgG/ml and 27.5 mg protein/ml), and rabbit anti-human albumin (5 mg IgG/ml and 12.1 mg protein/ml) were obtained from Cappel/Organon-Teknica (West Chester, PA). Additional rabbit anti-human IgG and anti-human IgM preparations (1 mg IgG/ml) were obtained from Fisher Scientific (Orangeburg, NY).

3. Results

Optimal concentrations of C′ or anti-IgG alone or a combination of both reagents to increase VZV Nt were established using HPZ serum.

3.1. Enhancement of neutralization with complement

The dilution endpoint of HPZ serum was increased 5-fold with 6–7 HLU of C′ (corresponding to 25–33% guinea pig serum, respectively, in the virus inoculum) (Table 1). These amounts of C′ produced 19 ± 5% (mean ± 1 S.D. in 4 experiments) reduction in VZV PFU in the absence of added human serum. Higher concentrations of guinea pig serum produced increased inactivation of VZV infectivity (56 and 35% VZV PFU losses with undiluted and 1 : 2 diluted guinea pig serum, respectively), and lower concentrations provided reduced Nt enhancement. Therefore, subsequent experiments used 7 HLU

Table 1
Enhancement of varicella-zoster virus neutralization activity of a postzoster serum using complement or anti-human IgG

Enhancement	VZV Neutralization (%) at reciprocal serum dilution [a]					
	50	250	1250	6250	31,250	156,250
Complement						
None	91	72	32	−13	26	ND [b]
7 [c]	99	99	91	15	21	17
6	ND	98	97	6	12	0
4	99	99	31	1	39	ND
7, heat-inactivated	ND	67	14	5	ND	ND
Rabbit anti-human IgG						
None	90	58	22	6	34	ND
0.5 [d]	ND	99	99	97	80	25
0.25	ND	99	99	83	34	ND
0.17	ND	99	98	85	37	ND
0.06	ND	99	97	48	−25	ND
0.03	99	98	73	23	23	ND

[a] Nt relative to control without serum. Dilution endpoints (highest dilution providing ⩾ 50% Nt) are underlined.
[b] ND, not determined.
[c] Complement concentration (HLU/reaction).
[d] Antibody amount (mg/reaction).

of C′ per reaction. Heat-inactivation (30 min at 56°C) of the guinea pig serum abolished the Nt enhancement.

3.2. Enhancement of neutralization with anti-IgG

VZV Nt titers increased with increasing amounts of rabbit anti-human IgG, up to the maximal tested amount of 0.5 mg per reaction (Table 1). Equivalent antibody amounts of rabbit anti-human IgM or anti-human albumin preparations did not enhance Nt (data not shown). Sheep and goat antibody preparations, which contained 2-fold higher antibody concentrations than the rabbit antiserum, produced increased reduction of VZV infectivity in the absence of added human serum (16, 38, and 76% reductions in VZV PFU for undiluted rabbit, goat, and sheep anti-human IgG preparations, respectively). Therefore, these sheep and goat preparations were not investigated further.

3.3. Enhancement of VZV neutralization with the combination of complement and anti-IgG

The relative amounts of Nt activity produced using combined enhancements for HPZ serum and VZIG were determined. VZV infectivity was typically reduced 24% following incubation with C′ and rabbit anti-IgG in the absence of added serum. The dilution endpoint of HPZ measured using the combined enhancements was 5-, 125-, and

625-fold greater than that measured using anti-IgG alone, C′ alone, or no enhancements, respectively (2 experiments), and ranged from 1:31,250 (69 ± 12% Nt (average ± 1 S.D.) in 26 separate tests) to 1:156, 250 (34 ± 14% Nt in 21 tests). The dilution endpoint of VZIG using combined enhancements (1:512,000) was twice that obtained using anti-IgG alone and 160-fold greater than the endpoint measured using no enhancements: enhancement with C′ alone was not determined. Based on these results for HPZ and VZIG, the combination of C′ (7 HLU/reaction) and anti-IgG (0.5 mg/reaction) was used for all subsequent experiments.

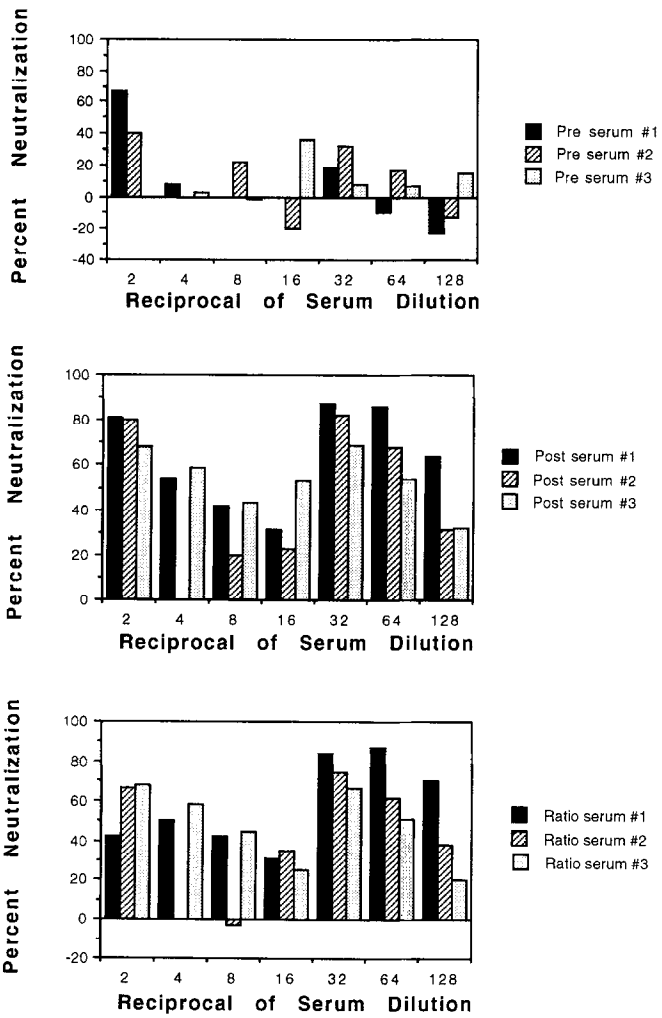

Fig. 1. Titration of VZV Nt antibodies in sera from 3 recipients of live varicella vaccine. Neutralization amounts were calculated relative to a 'no serum' control (method 1) for pre- and postvaccination sera and relative to the prevaccination sera (Ratio, method 2).

For 5 recipients of live varicella vaccine with weak anti-VZV responses (as measured by VARELISA), dilution endpoints of postvaccination sera were increased using combined enhancements by 13-, 32-, 32-, 50-, and 64-fold above those measured with no enhancements. Neutralization titers produced with different dilutions of sera from vaccinees were determined to attempt to identify a single dilution level providing maximal Nt. Use of combined anti-IgG and C′ (Fig. 1) in selected low-titered sera from vaccinees provided for maximal Nt titers at serum dilutions 1:32 or 1:64, coincident with the development of a visible precipitate. The lack of Nt activity using a negative control serum indicated that VZV infectivity was not affected simply by the formation of the precipitate.

3.4. Use of a single serum dilution to screen sera for antibodies to VZV

Based on these results, the VZV Nt antibody status of vaccinees was determined in a qualitative test at an optimal 1:64 serum dilution. Nt activities were determined relative to a 'no serum' control (method 1) or relative to the prevaccination serum (method 2). Repeat testing of a panel of 75 paired sera, representing primarily weak or non-responders to vaccination was performed to determine the standard deviation of the Nt activities. This analysis revealed that the use of a 50% Nt cutoff value was appropriate, providing a false-positive rate of $< 5\%$. A negative control serum produced $13 \pm 17\%$ Nt (average $\pm$ 1 S.D.) in 21 tests.

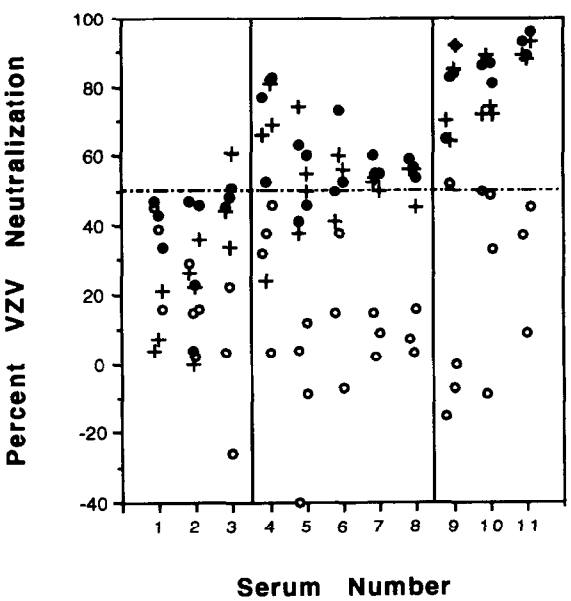

Fig. 2. Neutralization of VZV infectivity with a 1:64 dilution of sera from recipients of live varicella vaccine. Nt values were determined by method 1 for sera collected before (○) and 6 weeks after (●) vaccination, or by method 2 (+). Points above the dashed horizontal line represent significant amounts of Nt ($\geq 50\%$). Replicate points for each case represent Nt values measured in separate assays.

Table 2
Removal of varicella-zoster virus neutralizing antibodies from serum by incubation with varicella-zoster antigen

Serum	Percent neutralization after absoprtion with [a]		
	Buffer	VZV	MRC-5
V1	78	16	56
V2	64	17	50
HPZ	77	5	77

[a] V1 and V2 represent sets of paired sera form Varivax® recipients and HPZ is a human postzoster serum. Nt activity was calculated using method 2 (V1 and V2) or method 1 (HPZ).

Sera recorded as positive in at least 2 of 3 assays were assigned a final positive (Nt^+) score (false-positive rate $< 0.3\%$). Results from a panel of sera tested in multiple assays to demonstrate the reproducibility of the Nt amounts and concordance between Nt methods 1 and 2 are presented in Fig. 2.

Table 3
Lack of reaction of herpes simplex virus type 2 or cytomegalovirus antibodies in the enhanced neutralization assay for varicella-zoster virus antibodies

Serum [a]	Antivirus titer	Percent VZV neutralization at reciprocal dilution [b]					
		64	256	1024	4096	16,384	65,536
HSV-2							
1A	+	33	43	21	−1	4	ND [c]
1B	+ + +	33	35	22	13	11	ND
2A	+	ND	95	90	69	39	41
2B	+ + +	ND	91	73	38	18	42
3A	+	ND	96	89	65	55	28
3B	+ + +	ND	94	76	55	40	11
4A	+	ND	87	79	58	35	15
4B	+ + +	ND	89	86	43	30	8
CMV							
5A	< 2	ND	91	84	10	0	34
5B	⩾ 64	ND	95	75	34	27	36
6A	< 2	ND	95	94	57	36	3
6B	⩾ 64	ND	94	81	31	−2	0

[a] Samples 1–4 represent paired sera (originally designated serum pairs 3/4, 11/12, 19/20, and 43/44) showing significant boosts in anti-HSV-2 titers by immunoblot assay (A = pre and B = sera post-HSV flare up), and samples 5 and 6 represent paired sera (serum pairs 896/953 and 970/997) showing significant increases in anti-CMV antibody titers as measured by anti-C′ immunofluorescence assay (A = pre, b = postadministration of CMV vaccine). Immunoblot results of + indicate weak positive anti-HSV-2 signals and + + + indicate strong signals for anti-HSV-2 antibodies.

[b] Nt was measured relative to a control without serum (neutralization method 1). The dilution endpoints are underlined.

[c] ND, not determined.

Seroconversion failures were indicated for cases 1–3 by Nt amounts < 50% calculated by either Nt method. Weak seroconversions were identified for cases 4–8, where Nt shifted from negative to positive after vaccination. These seroconversions were confirmed using Nt calculation method 2. Strong seroconversions were measured for cases 9–11, where Nt values for postvaccination sera were maximal. Although Nt calculation methods 1 and 2 showed good agreement, the comparison of pre- and postvaccination sera in method 2 offered the advantage of an internal control to account for non-specific Nt and is therefore the more reliable measure of Nt.

3.5. Specificity of the enhanced Nt assay

Absorption of antibody from representative low-titered sera (at 1 : 64 dilution) or HPZ serum (1 : 1250 dilution) with VZV-infected MRC-5 cell extract, but not with a comparable amount of uninfected MRC-5 cell extract, significantly reduced the Nt amounts (Table 2), thus indicating the specificity of Nt antibodies for VZV.

A second demonstration of assay specificity was the absence of increased amounts of anti-VZV antibody in paired sera registering significant rises in levels of antibodies to HSV-2 or CMV (Table 3).

4. Discussion

Although both cell-mediated and humoral immunity are considered important for protection from varicella and zoster (reviewed by Gelb, 1990), antibody assays provide the most practical measurement of immune status to VZV. Virus Nt assays offer a surrogate of protection (Asano and Takahashi, 1978), measuring antibodies directed against viral glycoproteins (Keller et al., 1984; Kinchington et al., 1990; Vafai and Yang, 1991). A conventional Nt assay was too insensitive to measure antibodies developing in many individuals following vaccination with live attenuated varicella vaccine (Krah, unpublished data). This was consistent with the observation that antibody titers developing after vaccination are lower than those arising after natural infection (Dubey et al., 1988; Harper et al., 1990), but nevertheless protect against disease in most vaccinees (Provost et al., 1991; White et al., 1991, 1992).

Complement (Schmidt and Lennette, 1975) or anti-human IgG (Asano et al., 1982, 1983) supplements have been used individually to enhance the relatively inefficient Nt of VZV in vitro. In the present study, Nt enhancement with C′ alone was comparable to that previously reported (Schmidt and Lennette, 1975), whereas maximal enhancement with anti-IgG was 16-fold greater than that reported by Asano and colleagues (1982). The current study describes the first use of the combined enhancements to provide increased sensitivity, to avoid the Nt prozone effect observed with anti-IgG treatment (Asano et al., 1982) and to permit a more consistent measurement of Nt activity across serum dilutions.

Although the targets and mechanism of C′-mediated enhancement have been identified (Keller et al., 1986; Schmidt and Lennette, 1975; Forghani et al., 1990), the mechanism of Nt enhancement with anti-IgG has not been defined, but may involve

aggregation of antibody-sensitized virus or alteration of the binding affinity of primary antibody (Asano et al., 1982). A comparison of the VZV epitope maps generated using C′ (Forghani et al., 1990), anti-IgG, and their combination might serve to define the enhancement targets or mechanisms.

A qualitative test, using a single optimal serum dilution, was applied to determine the serum antibody status as an alternative to determining the serum neutralization dilution endpoint. This requires minimal volumes of sera (3 μl per assay), is amenable to the testing of large numbers of sera, and provides maximal sensitivity to measure antibodies in even low-titered sera. The concept of using a single serum dilution to measure antibodies was proposed for conventional Nt assays for anti-VZV antibodies (Takayama and Oya, 1981) and anti-IgG enhanced assays for anti-HSV antibodies (Holmes et al., 1985). In the present study, Nt of individual sera was judged relative to the 'no serum' control, and Nt in paired sera was assigned relative to the prevaccination control. Although both methods showed good agreement, the comparison of pre- and postvaccination sera offered the advantage of an internal control to account for non-specific inactivation of VZV infectivity.

The assay specificity was shown by the removal of Nt activity, following absorption of sera with VZV antigen and the absence of increased VZV Nt activity in paired sera showing significant antibody rises to HSV-2 or CMV. Additionally, the temporal relationship between pre- and postvaccination sera (6 weeks) limited the likelihood of false positives developing from concomitant infection with antigenically related herpesviruses (Schmidt, 1982; Davison et al., 1986; Emini et al., 1987; Giller et al., 1989). It is also noteworthy that similar Nt activity was measured using Oka or two 'wild-type' VZV isolates, thus indicating that the Nt activity was not unique to Oka VZV (D. Krah, unpublished data).

In summary, the enhanced Nt assay described now offers several advantages over previously described assays, including increased sensitivity and validation to measure antibodies at a single optimal serum dilution. This assay is proposed as a sensitive screening method to measure biologically active antibodies to VZV or to confirm the antibody status of sera producing equivocal results in other tests. The assay has been applied successfully to confirm seroconversions following vaccination with live varicella vaccine.

Acknowledgements

The authors gratefully acknowledge the expert technical assistance of G. Wallinger and R. Amin and thank T. Schofield for statistical analyses, S. Chatterjee, R. Whitley and S. Plotkin for human sera and HSV and CMV antibody assays, and C.J. White for human sera and helpful suggestions and critical review of the manuscript.

References

Asano, Y. and Takahashi, M. (1978) Studies on neutralization of varicella-zoster virus and serological follow-up of cases of varicella and zoster. Biken J. 21, 15–23.

Asano, Y., Albrecht, P., Stagno, S. and Takahashi, M. (1982) Potentiation of neutralization of varicella-zoster virus by antibody to immunoglobulin. J. Infect. Dis. 146, 524–529.

Asano, Y., Albrecht, P., Vujcic, L.K., Quinnan, G.V. and Takahashi, M. (1983) Evaluation of humoral immunity to varicella-zoster virus by an enhanced neutralization test and by the fluorescent antibody to membrane antigen test. Arch. Virol. 75, 225–228.

Caunt, A.E. and Shaw, D.G. (1969) Neutralization tests with varicella-zoster virus. J. Hyg. 67, 343–352.

Cremer, N.E., Cossen, C.K., Shell, G., Diggs, J., Gallo, D. and Schmidt, N.J. (1985) Enzyme immunoassay versus plaque neutralization and other methods for determination of immune status to measles and varicella-zoster viruses and versus complement fixation for serodiagnosis of infection with those viruses. J. Clin. Microbiol. 21, 869–874.

Davison, A.J., Edson, C.M., Ellis, R.W., Forghani, B., Gilden, D., Grose, C., Keller, P.M., Vafai, A., Wroblewska, Z. and Yamanishi, K. (1986) New common nomenclature for glycoprotein genes of varicella-zoster virus and their glycosylated products. J. Virol. 57, 1195–1197.

Dubey, L., Steinberg, S.P., LaRussa, P., Oh, P. and Gershon, A.A. (1988) Western blot analysis of antibody to varicella-zoster virus. J. Infect. Dis. 157, 882–888.

Emini, E.A., Luka, J., Armstrong, M.E., Keller, P.M., Ellis, R.W. and Pearson, G.R. (1987) Identification of an Epstein–Barr virus glycoprotein which is antigenically homologous to the varicella-zoster virus glycoprotein II and the herpes simplex virus glycoprotein. B. Virology 157, 552–555.

Forghani, B., Schmidt, N.J. and Dennis, J. (1978) Antibody assays for varicella-zoster virus: comparison of enzyme-immunoassay with neutralization, immune adherence hemagglutination and complement fixation. J. Clin. Microbiol. 8, 545–552.

Forghani, B., Dupuis, K.W. and Schmidt, N.J. (1990) Epitopes functional in neutralization of varicella-zoster virus. J. Clin. Microbiol. 28, 2500–2506.

Gelb, L.D. (1990) In: Fields, B.N., Knipe, D.M. et al. (Eds.), Virology, 2nd edn. Raven Press, New York, pp. 2011–2054.

Gelb, L.D., Dohner, D.E., Gershon, A.A., Steinberg, S.P., Waner, J.L., Takahashi, M., Dennehy, P.H. and Brown, A.E. (1987) Molecular epidemiology of live, attenuated varicella virus vaccine in children with leukemia and in normal adults. J. Infect. Dis. 155, 633–640.

Giller, R.H., Winistorfer, S. and Grose, C. (1989) Cellular and humoral immunity to varicella zoster virus glycoproteins in immune and susceptible human subjects. J. Infect. Dis. 160, 919–928.

Harper, D.R., Kangro, H.O. and Heath, R.B. (1990) Antibody responses in recipients of varicella vaccine assayed by immunoblotting. J. Med. Virol. 30, 61–67.

Holmes, P.J., Hallworth, J.A., Stocker, D.I. and Skinner, G.R.B. (1985) Augmentation of the neutralisation test for type I HSV: evidence of high representation of neutralising antibody in the adult community. Med. Microbiol. Immunol. 174, 167–175.

Keller, P.M., Neff, B.J. and Ellis, R.W. (1984) Three major glycoprotein genes of varicella-zoster virus whose products have neutralization epitopes. J. Virol. 52, 293–297.

Keller, P.M., Lonergan, K., Neff, B.J., Morton, D.A. and Ellis, R.W. (1986) Purification of individual varicella-zoster virus (VZV) glycoproteins gpI, gpII, and gpIII and their use in ELISA for detection of VZV glycoprotein-specific antibodies. J. Virol. Methods 14, 177–188.

Kinchington, P.R., Ling, P., Pensiero, M., Moss, B., Ruyechan, W.T. and Hay, J. (1990) The glycoprotein products of varicella-zoster virus gene 14 and their defective accumulation in a vaccine strain (Oka). J. Virol. 64, 4540–4548.

Kino, Y. and Minamishima, Y. (1993) Diagnosis of zoster and evaluation of varicella vaccine with a passive haemagglutination assay. Vaccine 11, 1151–1153.

Krah, D.L., Schofield, T.L. and Provost, P.J. (1990) Enhancement of varicella-zoster virus plaquing efficiency with an agarose overlay medium. J. Virol. Methods 27, 319–326.

Landry, M.L. and Ferguson, D. (1993) Comparison of latex agglutination test with enzyme-linked immunosorbent assay for detection of antibody to varicella-zoster virus. J. Clin. Microbiol. 31, 3031–3033.

Provost, P.J., Krah, D.L., Kuter, B.J., Morton, D.H., Schofield, T.L., Wasmuth, E.A., White, C.J., Miller, W.J. and Ellis, R.W. (1991) Antibody assays suitable for assessing immune responses to live varicella vaccine. Vaccine 9, 111–116.

Schmidt, N.J. (1982) Further evidence for common antigens in herpes simplex and varicella-zoster viruses. J. Med. Virol. 9, 27–36.

Schmidt, N.J. and Lennette, E.H. (1975) Neutralizing antibody responses to varicella-zoster virus. Infect. Immun. 12, 606–613.

Shehab, Z. and Brunell, P.A. (1983) Enzyme-linked immunosorbent assay for susceptibility to varicella. J. Infect. Dis. 148, 472–476.

Steinberg, S.P. and Gershon, A.A. (1991) Measurement of antibodies to varicella-zoster virus by using a latex agglutination test. J. Clin. Microbiol. 29, 1527–1529.

Takahashi, M., Okuno, Y., Otsuka, T., Osame, J., Takamizawa, A., Sasada, T. and Kubo, T. (1975) Development of a live attenuated varicella vaccine. Biken J. 18, 25–33.

Takayama, M. and Oya, A. (1981) A single serum dilution method for the quantitation of neutralizing antibodies to varicella-zoster virus. Biken J. 24, 109–118.

Vafai, A. and Yang, W. (1991) Neutralizing antibodies induced by recombinant virus expressing varicella-zoster virus gpIV. J. Virol. 65, 5593–5596.

Wasmuth, E.H. and Miller, W.J. (1990) Sensitive enzyme-linked immunosorbent assay for antibody to varicella-zoster virus using purified VZV glycoprotein antigen. J. Med. Virol. 32, 189–193.

White, C.J., Kuter, B.J., Hildebrand, C.S., Isganitis, K.L., Matthews, H., Miller, W.J., Provost, P.J., Ellis, R.W., Gerety, R.J. and Calandra, G.B. (1991) Varicella vaccine (Varivax®) in healthy children and adolescents: Results from clinical trials, 1987 to 1989. Pediatrics 87, 604–610.

White, C.J., Kuter, B.J., Ngai, A., Hildebrand, C.S., Isganitis, K.L., Patterson, C.M., Capra, A., Miller, W.J., Krah, D.L., Provost, P.J., Ellis, R.W. and Calandra, G.B. (1992) Modified cases of chickenpox following varicella vaccination: correlation of protection with antibody response. Pediatr. Infect. Dis. J. 11, 19–23.

Williams, V., Gershon, A. and Brunell, P.A. (1974) Serologic response to varicella-zoster membrane antigens measured by indirect immunofluorescence. J. Infect. Dis. 130, 669–672.