Appl Microbiol Biotechnol (2015) 99:4845–4853
DOI 10.1007/s00253-015-6602-5

METHODS AND PROTOCOLS

A monoclonal antibody-based VZV glycoprotein E quantitative assay and its application on antigen quantitation in VZV vaccine

Jian Liu[1] · Rui Zhu[2] · Xiangzhong Ye[3] · Lianwei Yang[3] · Yongmei Wang[1] · Yanying Huang[2] · Jun Wu[1] · Wei Wang[1] · Jianghui Ye[2] · Yimin Li[3] · Qinjian Zhao[2] · Hua Zhu[4] · Tong Cheng[1,2] · Ningshao Xia[1,2]

Received: 11 December 2014 / Revised: 7 April 2015 / Accepted: 20 April 2015 / Published online: 3 May 2015

Abstract Varicella-zoster virus (VZV) is a highly infectious agent that causes varicella and herpes zoster (HZ), which may be associated with severe neuralgia. Vaccination is the most effective way to reduce the burden of the diseases. VZV glycoprotein E (gE) is the major and most immunogenic membrane protein that plays important roles in vaccine efficacy. A quantitative assay for gE content is desirable for the VZV vaccine process monitoring and product analysis. In this study, 70 monoclonal antibodies (mAbs) were obtained after immunizing mice with purified recombinant gE (rgE). The collection of mAbs was well-characterized, and a pair of high-affinity neutralization antibodies (capture mAb 4A2 and detection mAb 4H10) was selected to establish a specific and sensitive sandwich enzyme-linked immunosorbent assay (ELISA) to quantify the native and recombinant gE. The detection limit of this assay was found to be 1.95 ng/mL. Furthermore, a reasonably good correlation between the gE content (as measured by the mAb-based quantitative ELISA) and the virus titer (as measured by the "gold standard" plaque assay) was observed when both assays were performed for tracking the kinetics of virus growth during cell culture. A total of 16 batches of lyophilized VZV vaccine were tested using the newly developed quantitative ELISA and classical plaque assay, demonstrating reasonably good correlation between gE content and virus titer. Therefore, this mAb-based gE quantitative assay serves as a rapid, stable, and sensitive method for monitoring viral antigen content, one additional quantitative method for VZV vaccine process and product characterization. This quantitative ELISA may also serve as a complementary method for virus titering.

Keywords Varicella-zoster virus (VZV) · VZV vaccine · Glycoprotein E · Sandwich ELISA · Virus titer

✉ Tong Cheng
tcheng@xmu.edu.cn

[1] State Key Laboratory of Molecular Vaccinology and Molecular Diagnostics, School of Life Sciences, Xiamen University, Xiamen 361102, China

[2] National Institute of Diagnostics and Vaccine Development in infectious diseases, School of Public Health, Xiamen University, Xiamen 361102, China

[3] Beijing Wantai Biological Pharmacy Enterprise, Beijing 102206, China

[4] Department of Microbiology and Molecular Genetics, New Jersey Medical School, Rutgers University, 225 Warren Street, Newark, NJ 070101, USA

Introduction

Varicella-zoster virus (VZV), a ubiquitous neurotropic human *alpha-herpesvirus*, is the causative agent for varicella and herpes zoster (HZ) (Arvin 2001). Varicella occurs as the primary infection and is a highly infectious disease. HZ is the result of the reactivation of the latent VZV infection of the nerve ganglia (Drolet et al. 2010). During zoster, the virus multiplies and spreads within the ganglion, causing neuronal necrosis and intense inflammation, a process that often results in severe neuralgia (Quinlivan and Breuer 2006). This may be associated with postherpetic neuralgia (PHN), a severe and the most common sequela of HZ (Dendouga et al. 2012). The incidence and severity of HZ and PHN increase with age, which is a burden for the aging society.

Currently, safe and efficient vaccines have been approved for the prevention of varicella and HZ. Clinical data have revealed that the varicella vaccine can reduce varicella incidence by >80 %, with corresponding reductions in hospitalizations and deaths (Guris et al. 2008; Schmid 2010). The

zoster vaccine markedly reduced morbidity due to HZ (approximately 50 %) and PHN (approximately 66 %) in persons aged >60 years (Oxman et al. 2005). An inactivated VZV vaccine has been assessed and was shown to stimulate the immune response in immunocompromised adults (Mullane et al. 2013). A vaccine based on a subunit of glycoprotein E (gE) has been evaluated and is in clinical trials (Dendouga et al. 2012; Lal et al. 2013). Inactivated vaccines and subunit vaccines might be potentially safer vaccines than current live-attenuated vaccines.

VZV encodes at least nine membrane proteins, and gE is the major and most essential membrane protein of VZV. Therefore, gE has attracted the interest of many researchers. VZV gE itself and its heterodimer forming with glycoprotein I (gI) play important roles in the replication of VZV in T cells and in skin and dorsal root ganglia (DRG) (Berarducci et al. 2009; Moffat et al. 2004; Zerboni et al. 2007). VZV gE is considered the major protective antigen and has been adopted as a potential subunit shingles vaccine (Dendouga et al. 2012; Leroux-Roels et al. 2012). Currently, Western blotting and immunofluorescence are the main ways to detect gE (Lenac Rovis et al. 2013; Olson et al. 1998). However, there is no quantitative assay, specifically an enzyme-linked immunosorbent assay (ELISA) technique, for determining the content of gE in vaccine formulations. In this study, recombinant gE (rgE) expressed by insect cells was purified to immunize BALB/c, and 70 gE monoclonal antibodies (mAbs) were collected. Through three rounds of pairing experiments, a specific and sensitive sandwich ELISA was established for the quantitative detection of native gE. In virus growth kinetics monitoring and lyophilized vaccine characterization, using rgE as the reference sample, the native gE content could be determined using the quantitative assay described above, which showed reasonably good correlation with the virus titer as determined by the plaque assay. Thus, this quantitative assay could be used as a sensitive and rapid method for monitoring gE content and might be used as a complementary method to assist the plaque assay for virus titer monitoring in VZV vaccine process and product characterization.

Materials and methods

Cells, virus, and VZV_{BAC}

Human acute retinal pigment epithelial cells (ARPE-19, purchased from ATCC, Manassas, VA, USA) were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS, PAA, Hyclone, Logan, UT, USA), 120 U/mL penicillin, 100 U/mL streptomycin (Invitrogen, Carlsbad, CA, USA), and 1 mM sodium pyruvate (Sigma-Aldrich, St. Louis, MO, USA). *Spodoptera frugiperda* (Sf21, purchased from Gibco, Grand Island, NY, USA) cells were routinely maintained in CCM_3 medium (Hyclone, Logan, UT, USA) containing 2 % FBS (PAA, Hyclone, Logan, UT, USA), 120 U/mL penicillin, and 100 U/mL streptomycin. Baculovirus (*Autographa californica* nuclear polyhedrosis virus, AcMNPV) was purchased from Clontech (Mountain View, CA, USA).

When ARPE-19 cells (routine culture in a 10-cm plate) reached 80 % confluence, they were infected with v-Oka-infected cells (with 90 % cytopathic effect) at a ratio of 1:10 and harvested every 12 h. Infected cells were harvested by rinsing the monolayer with phosphate-buffered saline (PBS), scraped into 1 mL of protection buffer (9 % sucrose, 25 mM histidine, 150 mM NaCl, pH 7.35), and stored at −80 °C. Aliquots were thawed to make cell-free viruses for the subsequent tests.

VZV_{BAC} was cloned from the p-Oka genome (GenBank No. AB097933.1) and maintained in the *Escherichia coli* strain DY380, and VZV_{BAC} was extracted with high purity using a large construct kit (Qiagen, Venlo, Netherlands) (Zhang et al. 2007).

Expression and purification of recombinant rgE

The gene-encoding truncate gE (1-537aa, GenBank No. AAY57748) was cloned from VZV_{BAC} into the transfer vector pFastBac (Invitrogen, Carlsbad, CA, USA) downstream of the polyhedron promoter. The recombinant baculovirus was constructed by following the protocol of the Bac-to-Bac system (Invitrogen, Carlsbad, CA, USA), and rgE was confirmed to be expressed in the medium of adherent cells using Western blotting with gE antibody 9C8 (Abcam, Cambridge, UK). For large-scale expression, the Sf21 cells were cultured in suspension using 500-mL conical flasks. Three days post-infection (multiplicity of infection, MOI=5), the medium was collected, rgE was purified, as described in the primary report (Kimura et al. 1997), and 10–20 mg of purified rgE was obtained per liter.

Monoclonal antibodies

MAbs against rgE were produced by following standard procedures. Specific pathogen-free BALB/c mice were injected subcutaneously with 100 μg of rgE emulsified in an equal volume of complete Freund's adjuvant (Sigma-Aldrich, St. Louis, MO, USA) and boosted at 2-week intervals with an equal amount of rgE in incomplete Freund's adjuvant (Sigma-Aldrich, St. Louis, MO, USA) twice. All mice were housed in the animal facility at the School of Life Science, Xiamen University. All studies were performed in accordance with institutionally approved protocols and the guidelines of the School of Life Sciences Guide for the Care and Use of Laboratory Animals. Spleen cells from immunized mice were fused with sp2/0 (myeloma cells) and selected for aminopterin

resistance. Hybridoma supernatant was tested for the presence of an antibody against rgE by indirect ELISA (100 ng rgE/well, with HRP-conjugated goat anti-mouse IgG as secondary antibody). Positive wells were cloned twice by limiting dilution, and 70 anti-gE mAbs were obtained at the end of the screening. Ascetic fluid produced from a single clone of positive cells was purified by protein A chromatography (GE Healthcare, Chalfont, Buckinghamshire, UK).

Pairing experiment base on antigen-capture ELISA for gE detection

v-Oka-infected cells (with 90 % cytopathic effect) were lysed with cell lysis buffer (20 mM TB7.5, 150 mM NaCl, 1 % Triton X-100), the supernatant was harvested to obtain native VZV gE, and the native gE was diluted with membrane protein dilution buffer (20 mM TB7.5, 150 mM NaCl, 0.2 % Triton X-100). All 70 anti-gE mAbs were coupled with horseradish peroxidase (HRP) and pre-coated on 96-well ELISA plates (200 ng/well), and an orthogonal experiment was performed to detect native VZV gE. In the first round of the pairing experiment, 4900 combinations (70×70) were used to detect native VZV gE with a dilution of 1:10, uninfected cell lysis was used as a negative control. MAb pairs with OD_{450} >3.0 (974 combinations) in the first pairing experiment were selected for the second pairing experiment to detect a higher dilution of native VZV gE (1:1000), and pairs with OD_{450} >3.0 were selected for the next pairing experiment. In the third pairing experiment, 26 pairs were tested with a twofold serial dilution of cell lysates, from 1:100 to 1:51,200 and the most sensitive pair of mAbs were determined.

Determination of VZV genome copies using fluorogenic polymerase chain reaction assay (TaqMan)

Virus-infected ARPE-19 cells were scraped into 1 mL of PBS, and 800 μL of cell suspension was used to extract the VZV genome using a genome extraction kit (TIANamp Micro DNA Kit, TIANGEN, Beijing, China). The remaining 200 μL was lysed to determine the content of gE, and the VZV genome was extracted by following the kit manual instructions. In the TaqMan-based polymerase chain reaction (PCR) assay (Hawrami and Breuer 1999), VZV_{BAC} was used as the reference sample to quantitatively determine the copies of the VZV genome extracted from virus-infected cells (Hawrami and Breuer 1999). The concentration of VZV_{BAC} used as the reference sample was 47.7 μg/mL and contained approximately 3.3×10^{11} copies/mL.

Neutralization assay using enzyme-linked immunosorbent spot assay

One day before the neutralization assay, the ARPE-19 cells were re-suspended and seeded in 24-well plates (NUNC, Roskilde, Denmark) at 50 % confluence. Then, 100 plaque forming units (PFU) of cell-free viruses was incubated with fivefold serially diluted mAbs in the protection buffer with or without a complement (1:10, *v*/*v*) at 37 °C for 1 h. Next, the virus-mAb-complement (or not) mixture was added to pre-seeded 24-well plates and incubated at 37 °C and 5 % CO_2 for 1 h. Fresh medium was used to replace the virus-mAb mixture. Each group was treated with the protection buffer alone, the virus with the complement or the virus with mAbs and denatured complement (56 °C for 30 min), and the viruses with protection buffer were used as control. The treatments were performed in triplicates. Three days post-infection, an enzyme-linked immunosorbent spot (Elispot) assay was performed, as described previously (Chen et al. 2014). The spot-forming cells were counted, and the titer that could neutralize half of the viruses was determined.

Virus titer determination using the plaque assay

In this study, the virus titer was calculated using the standard plaque assay with some minor modification. The plaque assay is widely regarded as the gold standard for quantification of infectious virus (Dulbecco 1952; Dulbecco and Weigle 1952; Krah et al. 1990). In detail, the ARPE-19 cells were seeded in six-well plates at 100 % confluence; a serial volume of cell-free viruses (100, 10, and 1 μL) was added in triplicate to each six-well plate. After incubating at 37 °C for 1 h, the supernatant and the PBS used to rinse the cells were collected for gE quantification. The cell layer was then covered with a layer of melted agar (*w*/*v*=0.8 %) containing DMEM. After solidification of the agar, the plates were incubated for 6–7 days at 37 °C. Cells were fixed by 4 % formaldehyde and dyed with 0.05 % neutral red. Then, the number of plaques was counted and the virus titer was determined.

Quantitative determination of gE content using the developed ELISA

In the study, purified rgE was used as a reference sample for quantification of native gE. The total gE content was determined from cell-free virus before incubation with cells in the plaque assay. The supernatant after incubation and PBS (2 mL/well) used to rinse the cells, which contains most gE that does not contribute to plaque formation, was collected and mixed. The cell-binding gE content was the deduction of gE in supernatant after incubation from total gE content.

Results

Development of a mAb-based sandwich ELISA to detect recombinant and native VZV gE

The extracellular domain (1-537aa) of gE was chosen to be expressed using the baculovirus expression system, the truncated gE bearing the native gE signal peptide. The gE gene was constructed into the baculovirus following the Bac-to-Bac protocol (Invitrogen, Carlsbad, CA, USA). The recombinant baculovirus genome was transfected into the insect cell line Sf21, and the expression was analyzed using Western blotting. Western blotting demonstrated that rgE was secreted into the medium. After purification, this rgE displayed two clear bands on performing SDS-PAGE; the larger band was approximately 61 kD, and the smaller one was approximately 50 kD. Both bands were verified by Western blotting with gE antibody 9C8 (Abcam, Cambridge, UK) (Kimura et al. 1998).

Purified rgE was used to immunize BALB/c mice, and 70 mAbs against gE were obtained. All 70 mAbs were labeled with HRP and coated on ELISA plates. An orthogonal experiment was performed to obtain pairs of mAbs to detect the native VZV gE. After three rounds of pairing experiments (Fig. 1a), we obtained a pair of mAbs (4A2/4H10-HRP) with the highest sensitivity and a low background reaction in detecting the native gE (Fig. 1b). To determine the sensitivity of the pair of antibodies in detecting the native gE, the VZV genome was used as a quantitative standard to quantify the native gE by using TaqMan-based PCR, as described previously (Hawrami and Breuer 1999). The 10-cm plate of virus-infected ARPE-19 cells used to prepare native gE contained approximately 1.21×10^9 copies of the VZV genome, and the pair of antibodies could detect native gE using as few as 4.7×10^3 copies of the VZV genome (Fig. 1c). The pair of mAbs was used to detect purified rgE, and we found that it could detect rgE at a concentration of 1.95 ng/mL (Fig. 1d). By using rgE as the reference sample (purity >95 %), we could quantitatively determinate the content of native gE in various samples.

Characteristics of the gE antibody 4A2 and 4H10

A highly sensitive and specific pair of mAbs was obtained to detect the VZV gE. They could be used to detect recombinant and native gE using Western blotting and immunofluorescence assay. VZV gE was identified as a membrane protein ranging from 70 to 100 kDa, expressed in the virions and virus-infected cell membranes. Western blotting demonstrated that both 4A2 and 4H10 could react with rgE (Fig. 2a) and v-Oka-infected cell lysate, but no band was detected in uninfected ARPE-19 cell lysate (Fig. 2b). In the immunofluorescence assay, a high fluorescence signal could be detected on the virus-infected cell membranes, and no signal was detected for the uninfected cells (Fig. 2c). These data indicated that 4A2 and 4H10 are specific for VZV gE.

Based on the Western blotting, the two antibodies were supposed to recognize the linear epitope located on gE. Furthermore, 15 amino acid (aa) peptides with 5 aa overlapping, covering the entire sequence of rgE, were used to react with the two antibodies to identify the peptides to which they bind (Table 1). Moreover, the result of the competition ELISA showed that the rgE-immunized mice serum (1:10) could block 4A2 (blocking rate 90.4 %) and 4H10 (blocking rate 36.6 %) binding to rgE (Table 1), and that the epitopes of 4A2 and 4H10 binding appeared to be immunodominant. Furthermore, the neutralization assay was performed with or without the complement, and it was found that both 4A2 and 4H10 are complement-dependent neutralizing antibodies; the neutralizing titer of 4A2 (1 mg/mL) is 1:2560, whereas that for 4H10 (1 mg/mL) is 1:640 (Table 1).

Virus growth kinetics monitoring using the constructed gE quantitative assay

VZV is highly cell associated in cell culture and is easy to be inactivated in the vaccine production process. Fast and accurate monitoring of virus growth kinetics is important to ensure reproducible viral cultivation and optimize vaccine production. However, the conventional method to monitor the process is the plaque assay, which is laborious and time-consuming. Therefore, efficient and rapid methods are greatly needed to assist the classical plaque assay for virus titer monitoring in the cell culture process. In the study, the total gE content and cell-binding gE content were compared with the virus titer. Twenty-four plates of cells were infected with v-Oka at the same MOI (MOI=0.1), and three plates were scraped once with 1-mL protection buffer per plate, every 12 h, to prepare cell-free virus. Titer of the cell-free virus was determined using the plaque assay, while total gE and cell-binding gE content was determined using the quantitative assay. The gE content was compared with the virus titer, and the correlation between the methods was analyzed.

As shown in Fig. 3, the content of total gE and cell-binding gE content was increasing with an increase in the infectious VZV formation during the cell culture process (Fig. 3a, c). A reasonably good correlation was demonstrated between total gE content and virus titer (r=0.829, Fig. 3b), and cell-binding gE content showed higher correlation coefficient with virus titer than total gE content (r=0.950, Fig. 3d). In the late stage of cell culture, virus titer decreased rapidly, while decrease of total gE protein was delayed (Fig. 3a). Exclusion of gE not contributing to virus infection improved the correlation between gE content s.

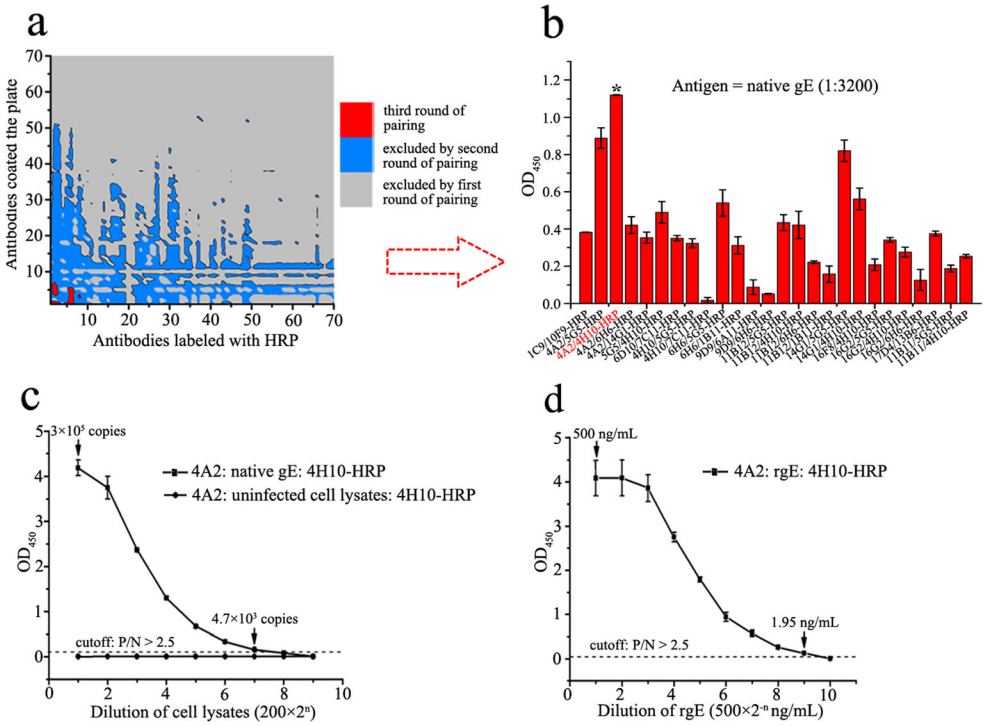

Fig. 1 Development of a sandwich ELISA for detecting native and recombinant gE. **a** Pairing experiment. Three rounds of pairing experiment were performed with serial dilution of gE. In the first round of pairing experiment, 4900 combinations (70×70) were tested with virus-infected ARPE-19 cell lysates at a 1:10 dilution, and 974 pairs (colored *blue* and *red*) of mAbs with OD_{450} greater than 3.0 were selected for the next pairing experiment with a higher dilution (1:1000). In the third round of the experiment, 26 pairs of mAbs (colored *red*) with OD_{450} greater than 3.0 in the second round of pairing experiment were tested with a twofold serial dilution of cell lysates from 1:100 to 1:51,200. **b** The pair of 4A2/4H10-HRP (one of the 26 pairs colored in red in the third round of pairing experiments) showed the highest sensitivity in the pairing experiment. **c** Detection of v-Oka-infected ARPE-19 cell lysates (harvested 3 days post-infection, MOI=0.2) and uninfected ARPE-19 cell lysates. The pair of mAbs (4A2/4H10-HRP) could detect up to 4.7×10^3 copies of the VZV genome in the v-Oka-infected ARPE-19 cell lysates (cutoff P/N >2.5), while it could not react with uninfected ARPE-19 cell lysates. **d** Detection of rgE. The pair of mAbs (4A2/4H10-HRP) could detect purified rgE at a concentration of 1.95 ng/mL (cutoff P/N >2.5)

Characterization of lyophilized varicella vaccine using the constructed gE quantitative assay

The current varicella vaccine is live attenuated, and the classical approach for the characterization of lyophilized varicella vaccine is to determine the number of plaque forming units (PFU) per dose by the plaque assay. As the gE content has reasonably good correlation with the virus titer in cell culture process, the same strategy was used to evaluate different batches of lyophilized varicella vaccine. Sixteen batches of lyophilized varicella vaccine (Wantai Biological Pharmacy Enterprise, Beijing, China) were used in the study. Each dose of lyophilized vaccine was solubilized with 1 mL of PBS, and serial volumes of aliquots (50 and 10 μL) were added in triplicate to each six-well plate to determine the virus titer using the plaque assay. The total gE content and cell-binding gE content were determined, and the correlation between the methods was analyzed. The correlation coefficient between total gE content and virus titer was 0.755 (Fig. 4a), while correlation coefficient between cell-binding gE content and virus titer was 0.855 (Fig. 4c). Furthermore, the ratio of gE binding to cells to total gE was analyzed and appeared quite steady; it fluctuated between 50 and 65 % (Fig. 4b). It might be another indicator to characterize the lyophilized varicella vaccine.

Discussion

VZV infection may cause two different symptoms named varicella and HZ, and varicella is one of the most contagious diseases known. Vaccines specific to these two diseases are available, and the current data show that the varicella vaccine and zoster vaccine have achieved significant vaccine efficacy (Nguyen et al. 2005; Oxman et al. 2005; Seward et al. 2002; Zhou et al. 2005). Combined with the discovery of VZV skin- or neurotropic factors, new virus strains with deleted ORFs might be used as potential safer live-attenuated vaccine (Che et al. 2008; Selariu et al. 2012; Zhang et al. 2010). Inactivated zoster vaccine as a safer form of vaccine has been evaluated in immunocompromised adults in clinical trials and showed promising prospects (Mullane et al. 2013). The gE protein is considered the major protective antigen and can stimulate both humoral and cellular immunity (Dendouga et al. 2012; Lal

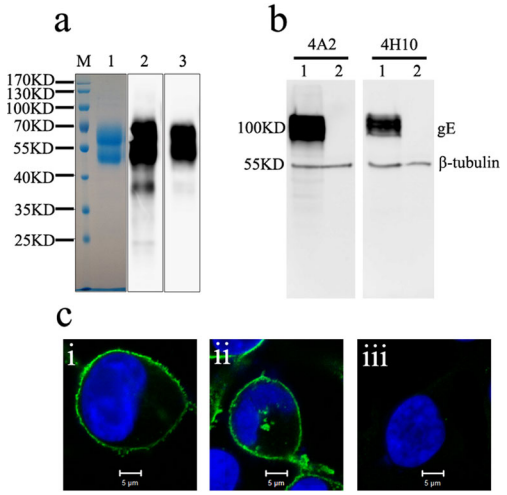

Fig. 2 Identification of 4A2 and 4H10. **a** Identification of 4A2 and 4H10 with purified rgE using Western blotting. Purified rgE was subjected to 12 % SDS-PAGE (*lane 1*). The protein was transferred to a nitrocellulose membrane, incubated with antibodies 4A2 (*lane 2*) and 4H10 (*lane 3*), respectively, and then incubated with a goat anti-mouse secondary antibody conjugated with horseradish peroxidase (Sigma-Aldrich, St. Louis, MO, USA). SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Fisher, Waltham, MA, USA) was used, and the signal was collected using ImageQuant LAS 4000 mini (GE Healthcare, Chalfont, Buckinghamshire, UK). **b** Identification of 4A2 and 4H10 with native gE using Western blotting. Western blotting was performed as described above, and β-tubulin (7B9, Abcam, Cambridge, UK) was used as the internal reference. *Lane 1* v-Oka-infected ARPE-19 cell lysates; *lane 2* uninfected ARPE-19 cell lysates. **c** Cellular localization of VZV gE revealed by 4A2 and 4H10. v-Oka-infected and v-Oka-uninfected ARPE-19 cells (*iii*) were fixed, permeated, and incubated with 4A2 (*i*) and 4H10 (*ii*), followed by incubation with a FITC-conjugated goat anti-mouse secondary antibody (*green*). The nuclei were stained with DAPI (*blue*). The image magnification was ×630

et al. 2013; Leroux-Roels et al. 2012). The result of a pivotal phase III study showed that gE subunit vaccine could reduce the risk of shingles by 97.2 % in adults aged 50 years and older compared to placebo (GlaxoSmithKline 2014). However, there is no quantitative assay, specifically ELISA technique, for determining the content of gE in vaccine formulations that has been reported.

In the present study, rgE was expressed in the baculovirus expression system; it was well-modified and secreted into the culture medium. A collection of 70 mAbs was obtained using BALB/c immunized with purified rgE, three pairing experiments were performed, and a highly sensitive quantitative assay was established based on the sandwich ELISA technique to determine the gE content. The assay could detect the native gE in rapid and high throughput manner. Furthermore, the gE content demonstrated a reasonably good correlation with the virus titer in the cell culture process monitoring and different batches of lyophilized varicella vaccine characterization. Thus, the constructed gE quantitative assay could be used as a rapid and sensitive method for gE quantitation and might be used as a complementary method to assist plaque assay in vaccine production and development. Comparing with 6–7 days needed for the plaque assay, which is the classical and most frequently used method for the detection of virus titer, the detection time of ELISA method was significantly shortened to several hours.

Table 1 Characteristics of 4A2 and 4H10

	Subtype	Epitope (linear)	Block ratio by rgE-immunized mice serum (1:10) (%)	Neutralizing titer (complement 1:10 *v/v*)
4A2	IgG1	SAQEDLGDD TGIHVI	90.40	1:2560
4H10	IgG1	FHMWNYHS HVFSVGD	36.60	1:640

VZV ORF68 is the late gene which encodes the major membrane protein gE. The expression level of gE protein increases with infectious virus formation in the cell culture process. In the study, reasonably good correlation between gE content by the mAb-based ELISA and virus titer by plaque assay was observed with a moderate correlation coefficients (r=0.829, 0.950, 0.755, and 0.855; Figs. 3 and 4). The moderate correlation is largely due to the variability of classical plaque assay. The assay variation could be as high as 33 % as reported with VZV (Krah et al. 1990). A reported work showed that the correlation coefficient between quantitative real-time PCR and plaque assay in detecting yellow fever virus was 0.88 (r=0.88) (Bae et al. 2003). Another recent publication on dengue virus reported reasonably good agreement between Elispot assay and classical plaque reduction assay with a correlation coefficient r=0.82 (Liu et al. 2012). Therefore, the VZV gE content measured by the quantitative assay was reasonably correlated with virus titer determined by the plaque assay in the study. Furthermore, the constructed mAb-based sandwich assay could quantitate all forms of gE protein including gE protein not contributing to infection, while the plaque assay has been used to quantify infectious virus. The presence of gE in cell fragments or noninfectious virus particles might be one of most important factors to influent the correlation between gE content and virus titer. Therefore, an additional version of the gE quantitation was developed to partially remove the influence of the gE components not contributing to the virus plaque formation. In the study, the correlation coefficient of r=0.755 or 0.829 for the correlation between plaque assay and total gE assay was further increased to r=0.855 or 0.950 when the cell-binding gE content (instead of the total) was used for correlation analysis (Figs. 3 and 4).

The 4A2 and 4H10 used in the constructed method were well-characterized, and their application might be expanded beyond their use in quantifying the content of gE during the vaccine production process. The pair of mAbs was screened from 4900 combinations and is the most sensitive and specific

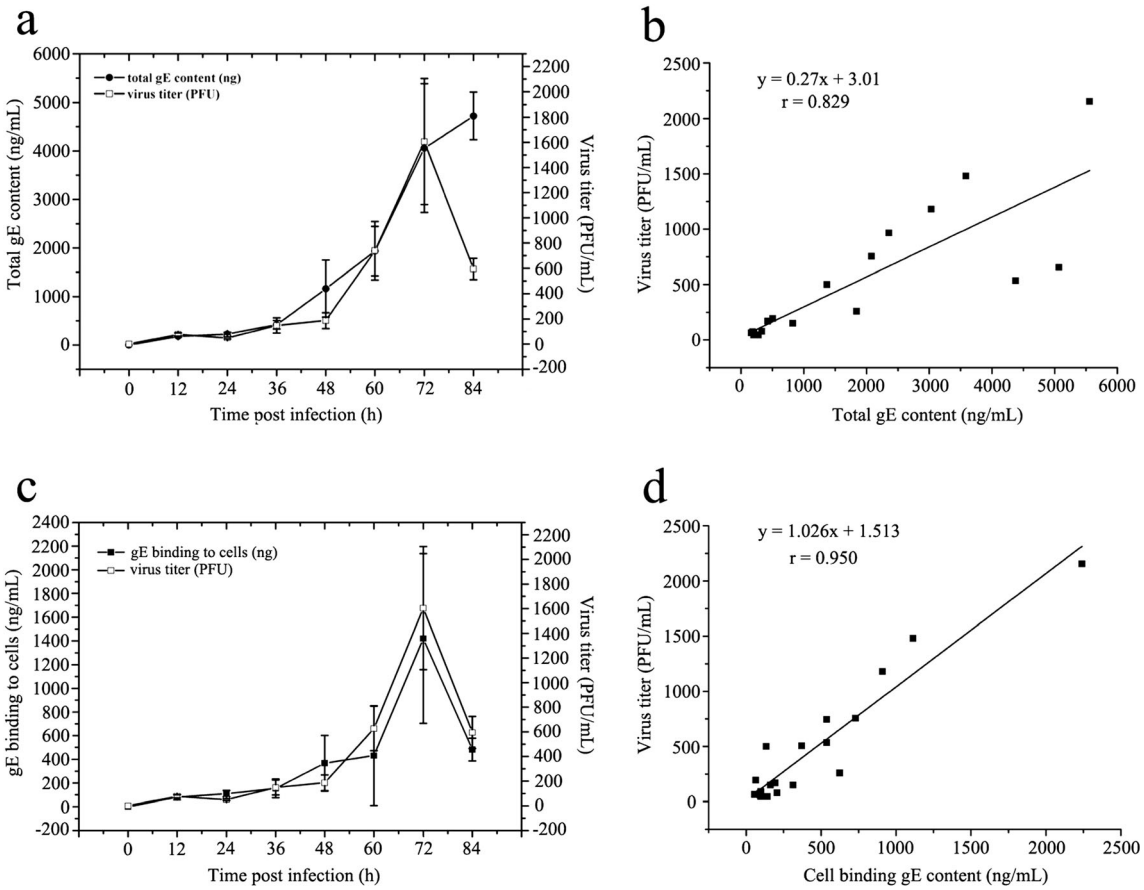

Fig. 3 Comparison of the gE content with virus titer during cell culture process. Twenty-four plates (10 cm) of ARPE-19 cells were infected with v-Oka at the same MOI (MOI=0.1); every 12 h, three plates of the virus-infected cells were scraped in protection buffer (9 % sucrose, 25 mM histidine, 150 mM NaCl) with 1 mL/plate and frozen at −80 °C. The cell-free viruses were obtained by thawing and low-speed centrifugation (400 g, 10 min). The titer of cell-free virus was determined using the plaque assay, while total gE content and cell-binding gE content were determined using the quantitative ELISA. Total gE content and cell-binding gE content were compared with virus titer during the cell culture process. **a** The content of total gE increased with virus titer during the cell culture process; in the late stage of culture, virus titer dropped rapid, while the decrease of total gE protein was delayed. **b** The correlation between total gE content and virus titer was analyzed, and the correlation coefficient (*r*) was 0.829. **c** The cell-binding gE content and virus titer increased with the same trend in the process of cell culture. **d** The correlation between cell-binding gE content and virus titer was analyzed, and the correlated coefficient (*r*) is 0.950. The correlation between gE content and virus titer has been improved after partial exclusion of gE not contributing to plaque formation

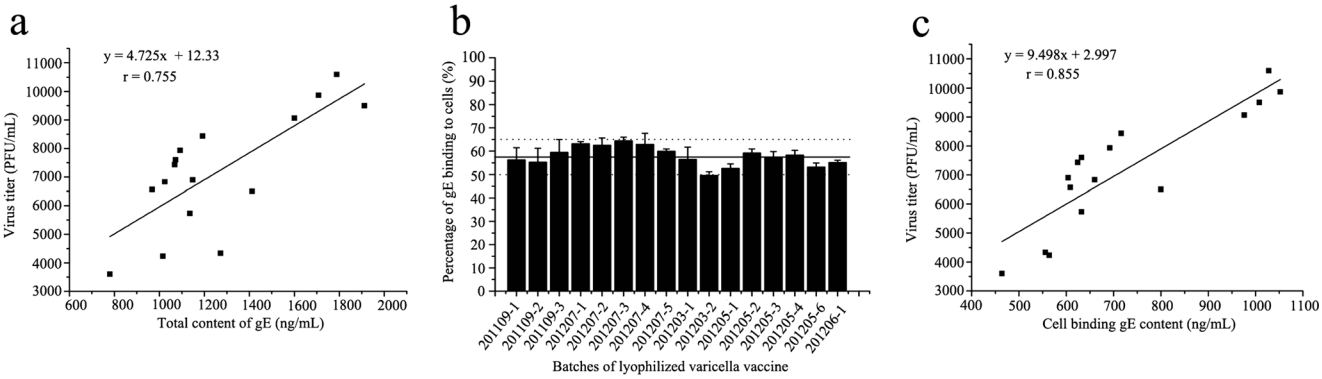

Fig. 4 Comparison of the gE content with virus titer in lyophilized varicella vaccine characterization. Sixteen batches of lyophilized varicella vaccine were solubilized with 1 mL of PBS per dose. Aliquots (50 and 10 μL) were added in triplicate to each six-well plate to determine the virus titer using the plaque assay. Total gE content, cell-binding gE content, and virus titer in the 10-μL group were determined with the quantitative assay or the plaque assay. The correlation between gE content and virus titer was analyzed. **a** Analysis of correlation between total gE content and virus titer, and the correlation coefficient (*r*) was 0.755. **b** The ratio of gE binding to cells to total gE of the 16 batches of lyophilized varicella fluctuated between 50 and 65 %. **c** Correlation between cell-binding gE content and virus titer, the correlation coefficient (*r*) was 0.872

pair. Several studies are required to detect VZV infection in tissues and organs and thus need to determine the upregulation or downregulation of gE among various samples. This purpose may be achieved by lysing the cells or tissues with a lysis buffer and detecting gE using the quantitative assay described above. Additionally, the conservation of the two antibody epitopes was analyzed by BLAST comparison using the nucleotide sequence database; the conservation of the 4A2 epitope was found to be 100 % among the strains or isolations of VZV, whereas the conservation of 4H10 was found to be 93 %. Therefore, the quantitative assay could be used to quantitatively determine the gE content of different strains of VZV. Furthermore, the two antibodies could neutralize the virus in the presence of a complement, and the rgE-immunized mice serum could block the antibodies binding to rgE. The epitopes of the two antibodies might be major neutralized sites and play an important role in vaccine efficacy; therefore, the pair of mAbs could be used to monitor important epitopes and improve vaccine efficacy.

Acknowledgments This work was supported by a grant from the National High Technology Research and Development Program of China (No. 2012AA02A408) and National Science and Technology Major Project of Infectious Diseases (No. 2012ZX10004503-005). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Arvin AM (2001) Varicella-zoster virus: molecular virology and virus-host interactions. Curr Opin Microbiol 4(4):442–9

Bae HG, Nitsche A, Teichmann A, Biel SS, Niedrig M (2003) Detection of yellow fever virus: a comparison of quantitative real-time PCR and plaque assay. J Virol Methods 110(2):185–91

Berarducci B, Rajamani J, Reichelt M, Sommer M, Zerboni L, Arvin AM (2009) Deletion of the first cysteine-rich region of the varicella-zoster virus glycoprotein E ectodomain abolishes the gE and gI interaction and differentially affects cell-cell spread and viral entry. J Virol 83(1):228–40. doi:10.1128/JVI.00913-08

Che X, Reichelt M, Sommer MH, Rajamani J, Zerboni L, Arvin AM (2008) Functions of the ORF9-to-ORF12 gene cluster in varicella-zoster virus replication and in the pathogenesis of skin infection. J Virol 82(12):5825–34. doi:10.1128/JVI.00303-08

Chen L, Liu J, Wang W, Ye J, Wen L, Zhao Q, Zhu H, Cheng T, Xia N (2014) Development of a varicella-zoster virus neutralization assay using a glycoprotein K antibody enzyme-linked immunosorbent spot assay. J Virol Methods 200C:10–14. doi:10.1016/j.jviromet.2014.01.014

Dendouga N, Fochesato M, Lockman L, Mossman S, Giannini SL (2012) Cell-mediated immune responses to a varicella-zoster virus glycoprotein E vaccine using both a TLR agonist and QS21 in mice. Vaccine 30(20):3126–35. doi:10.1016/j.vaccine.2012.01.088

Drolet M, Brisson M, Schmader KE, Levin MJ, Johnson R, Oxman MN, Patrick D, Blanchette C, Mansi JA (2010) The impact of herpes zoster and postherpetic neuralgia on health-related quality of life: a prospective study. CMAJ : Can Med Assoc J = journal de l'Association medicale canadienne 182(16):1731–6. doi:10.1503/cmaj.091711

Dulbecco R (1952) Production of plaques in monolayer tissue cultures by single particles of an animal virus. Proc Natl Acad Sci U S A 38(8):747–52

Dulbecco R, Weigle JJ (1952) Inhibition of bacteriophage development in bacteria illumination with visible light. Experientia 8(10):386–7

GlaxoSmithKline (2014) Pivotal phase III study of GSK shingles candidate vaccine meets its primary endpoint. Publisher. http://www.gsk.com/en-gb/media/press-releases/2014/pivotal-phase-iii-study-of-gsk-shingles-candidate-vaccine-meets-its-primary-endpoint/ Accessed 19 February 2015

Guris D, Jumaan AO, Mascola L, Watson BM, Zhang JX, Chaves SS, Gargiullo P, Perella D, Civen R, Seward JF (2008) Changing varicella epidemiology in active surveillance sites—United States, 1995–2005. J Infect Dis 197(Suppl 2):S71–5. doi:10.1086/522156

Hawrami K, Breuer J (1999) Development of a fluorogenic polymerase chain reaction assay (TaqMan) for the detection and quantitation of varicella zoster virus. J Virol Methods 79(1):33–40

Kimura H, Straus SE, Williams RK (1997) Varicella-zoster virus glycoproteins E and I expressed in insect cells form a heterodimer that requires the N-terminal domain of glycoprotein I. Virology 233(2):382–91. doi:10.1006/viro.1997.8625

Kimura H, Straus SE, Williams RK (1998) Baculovirus expression, purification, and properties of varicella-zoster virus gE, gI, and the complex they form. J Infect Dis 178(Suppl 1):13–15

Krah DL, Schofield TL, Provost PJ (1990) Enhancement of varicella-zoster virus plaquing efficiency with an agarose overlay medium. J Virol Methods 27(3):319–25

Lal H, Zahaf T, Heineman TC (2013) Safety and immunogenicity of an AS01-adjuvanted varicella zoster virus subunit candidate vaccine (HZ/su): a phase-I, open-label study in Japanese adults. Hum Vaccin Immunother 9(7):1425–9. doi:10.4161/hv.24269

Lenac Rovis T, Bailer SM, Pothineni VR, Ouwendijk WJ, Simic H, Babic M, Miklic K, Malic S, Verweij MC, Baiker A, Gonzalez O, von Brunn A, Zimmer R, Fruh K, Verjans GM, Jonjic S, Haas J (2013) A comprehensive analysis of varicella zoster virus proteins using a new monoclonal antibody collection. J Virol. doi:10.1128/JVI.00407-13

Leroux-Roels I, Leroux-Roels G, Clement F, Vandepapeliere P, Vassilev V, Ledent E, Heineman TC (2012) A phase 1/2 clinical trial evaluating safety and immunogenicity of a varicella zoster glycoprotein e subunit vaccine candidate in young and older adults. J Infect Dis 206(8):1280–90. doi:10.1093/infdis/jis497

Liu L, Wen K, Li J, Hu D, Huang Y, Qiu L, Cai J, Che X (2012) Comparison of plaque- and enzyme-linked immunospot-based assays to measure the neutralizing activities of monoclonal antibodies specific to domain III of dengue virus envelope protein. Clin Vaccine Immunol 19(1):73–8. doi:10.1128/CVI.05388-11

Moffat J, Mo C, Cheng JJ, Sommer M, Zerboni L, Stamatis S, Arvin AM (2004) Functions of the C-terminal domain of varicella-zoster virus glycoprotein E in viral replication in vitro and skin and T-cell tropism in vivo. J Virol 78(22):12406–15. doi:10.1128/JVI.78.22.12406-12415.2004

Mullane KM, Winston DJ, Wertheim MS, Betts RF, Poretz DM, Camacho LH, Pergam SA, Mullane MR, Stek JE, Sterling TM, Zhao Y, Manoff SB, Annunziato PW (2013) Safety and immunogenicity of heat-treated zoster vaccine (ZVHT) in immunocompromised adults. J Infect Dis. doi:10.1093/infdis/jit344

Nguyen HQ, Jumaan AO, Seward JF (2005) Decline in mortality due to varicella after implementation of varicella vaccination in the United States. N Engl J Med 352(5):450–8. doi:10.1056/NEJMoa042271

Olson JK, Santos RA, Grose C (1998) Varicella-zoster virus glycoprotein gE: endocytosis and trafficking of the Fc receptor. J Infect Dis 178(Suppl 1):S2–6

Oxman MN, Levin MJ, Johnson GR, Schmader KE, Straus SE, Gelb LD, Arbeit RD, Simberkoff MS, Gershon AA, Davis LE, Weinberg A, Boardman KD, Williams HM, Zhang JH, Peduzzi PN, Beisel CE,

Morrison VA, Guatelli JC, Brooks PA, Kauffman CA, Pachucki CT, Neuzil KM, Betts RF, Wright PF, Griffin MR, Brunell P, Soto NE, Marques AR, Keay SK, Goodman RP, Cotton DJ, Gnann JW Jr, Loutit J, Holodniy M, Keitel WA, Crawford GE, Yeh SS, Lobo Z, Toney JF, Greenberg RN, Keller PM, Harbecke R, Hayward AR, Irwin MR, Kyriakides TC, Chan CY, Chan IS, Wang WW, Annunziato PW, Silber JL (2005) A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N Engl J Med 352(22): 2271–84. doi:10.1056/NEJMoa051016

Quinlivan M, Breuer J (2006) Molecular studies of varicella zoster virus. Rev Med Virol 16(4):225–50. doi:10.1002/rmv.502

Schmid DS (2010) Varicella-zoster virus vaccine: molecular genetics. In: Abendroth A, Arvin AM, Moffat JF (eds). Springer, pp 323-340

Selariu A, Cheng T, Tang Q, Silver B, Yang L, Liu C, Ye X, Markus A, Goldstein RS, Cruz Cosme RS, Lin Y, Wen L, Qian H, Han J, Dulal K, Huang Y, Li Y, Xia N, Zhu H (2012) ORF7 of varicella zoster virus is a neurotropic factor. J Virol. doi:10.1128/JVI.00128-12

Seward JF, Watson BM, Peterson CL, Mascola L, Pelosi JW, Zhang JX, Maupin TJ, Goldman GS, Tabony LJ, Brodovicz KG, Jumaan AO, Wharton M (2002) Varicella disease after introduction of varicella vaccine in the United States, 1995-2000. JAMA : J Am Med Assoc 287(5):606–11

Zerboni L, Reichelt M, Jones CD, Zehnder JL, Ito H, Arvin AM (2007) Aberrant infection and persistence of varicella-zoster virus in human dorsal root ganglia in vivo in the absence of glycoprotein I. Proc Natl Acad Sci U S A 104(35):14086–91. doi:10.1073/pnas.0706023104

Zhang Z, Rowe J, Wang W, Sommer M, Arvin A, Moffat J, Zhu H (2007) Genetic analysis of varicella-zoster virus ORF0 to ORF4 by use of a novel luciferase bacterial artificial chromosome system. J Virol 81(17):9024–33. doi:10.1128/JVI.02666-06

Zhang Z, Selariu A, Warden C, Huang G, Huang Y, Zaccheus O, Cheng T, Xia N, Zhu H (2010) Genome-wide mutagenesis reveals that ORF7 is a novel VZV skin-tropic factor. PLoS Pathog 6: e1000971. doi:10.1371/journal.ppat.1000971

Zhou F, Harpaz R, Jumaan AO, Winston CA, Shefer A (2005) Impact of varicella vaccination on health care utilization. JAMA : J Am Med Assoc 294(7):797–802. doi:10.1001/jama.294.7.797