Varicella-Zoster Virus Subunit Vaccine

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Abstract: Varicella-zoster virus (VZV) causes chickenpox (Varicella) in children and shingles (Zoster) in elderly and immunosuppressed individuals. An attenuated VZV vaccine has been approved for general immunization to prevent varicella and zoster in the United States. Although this vaccine provides a high degree of protection against virus infection, the virus becomes latent in dorsal root ganglia and will reactivate to produce zoster. Therefore, there is a need for developing additional VZV vaccines that are capable of eliciting immune response to the virus but will not establish viral latency. Our studies have been focused on the development of a truncated secretory VZV glycoprotein (VZVgE) subunit vaccine. The results from our studies have shown that the VZV subunit vaccine: (1) elicits the induction of neutralizing antibodies in animals as well as in humans; and (2) stimulates the induction of VZVgE-specific antibodies in VZV-seropositive human mononuclear cells. Such a VZV glycoprotein antigen, therefore, may have the potential to be used as a candidate VZV glycoprotein subunit vaccine for prevention of primary VZV infection (Varicella) or boosting immune response against VZV reactivation (Zoster) in adults, the elderly and immunosuppressed individuals.

Introduction

Varicella-zoster virus (VZV), a member of the human herpesvirus family, causes childhood chickenpox (varicella), becomes latent in dorsal root ganglia and reactivates years later to produce shingles (zoster) in immunocompromised and aging individuals. Complications associated with primary infection with VZV (chickenpox) and VZV reactivation (shingles) are shown in Table 1 [1-3]. Prior to the adoption of Varicella vaccine, there were an estimated 4 million cases of varicella and 1.2 million cases of zoster each year in the United States [4-6].

Viruses isolated from clinical cases of varicella and zoster have identical antigenic and biological properties indicating that there is only one serotype of VZV [7, 8]. VZV is morphologically similar to herpesviruses and classified as a member of *alphaherpesviridae* [4, 9]. VZV consists of a DNA-containing core in a protein capsid, called a nucleocapsid, which is surrounded by an outer membrane. Morphogenesis of VZV in infected cells also appears to be similar to other herpesviruses [10]. After virus penetration, the viral envelope is presumably removed in the cytoplasmic vacuoles and the viral DNA becomes associated with the nucleus. Viral DNA and proteins are synthesized and nucleocapsids measuring approximately 100 nm are assembled in the nucleus. Nucleocapsids acquire an envelope from the inner membrane during passage to the cytoplasm. Pleomorphic particles measuring approximately 200 nm are released to the exterior of the cell by exocytosis [11].

The replication cycle of VZV in tissue culture cells is longer than that of herpes simplex virus (HSV). HSV DNA synthesis begins at 2 hr. postinfection (p.i.), peaks at 4 hr. p.i., and infectious virions are first detected in the infected cells at 13 hr. p.i. [12]. VZV DNA synthesis, however, begins at 10 hr. p.i., peaks at 18 hr. p.i., and the first progeny virus appears in the infected cells at about 29 hr. p.i. [13].

VZV, contains a linear double-stranded DNA molecule of 124,884 base pairs (bp). The entire VZV DNA has been sequenced [14] and is predicted to encode 71 proteins, including five major viral glycoproteins designated VZV gE, VZV gB, VZV gH, VZV gI and VZV gC [15-17; 1993 International Herpersvirus Workshop]. These glycoproteins are the major virus-specific components found on the viral envelope and VZV-infected cell membrane.

VZV Vaccine

An attenuated VZV vaccine (Oka/Merck) has been approved by the Food and Drug Administration (FDA) for general immunization to prevent varicella and zoster in the United States [18, 19]. The vaccine elicits both humoral and cell-mediated immunity [20, 21]. However, although this vaccine provides a high degree of protection against varicella, one concern regarding its use has been the duration of protection after immunization [22]. In addition, similar to natural infection, attenuated varicella vac-

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cine becomes latent in dorsal root ganglia and will reactivate to produce zoster [23, 24]. Therefore, there is a need for developing additional VZV vaccines (e.g., subunit vaccine) that are capable of eliciting immune responses to virus infection but that will not establish virus latency. Subunit vaccines have been prepared for a number of viruses [for review see 25 and 26]. Vaccine development for these viruses has centered mainly on viral glycoproteins, since these proteins elicit both humoral and cell-mediated immune responses following virus infection. Since VZV gE (previously designated gpI) is highly immunogenic and immune response to this glycoprotein is the first to appear following primary infection (varicella) and VZV reactivation (zoster), our studies have been focused on the development of a VZV gE subunit vaccine.

VZV gE (gpI)

VZV gE is the most abundant and immunogenic of the VZV glycoproteins. This viral glycoprotein elicits the formation of complement-dependent neutralizing antibodies and also cell-mediated immunity [15, 27]. The VZV glycoprotein gI (previously designated gpIV) and gE are encoded by VZV genes 67 and 68, respectively, which are located within the SalI-I DNA fragment (Fig. 1) of the unique short sequences (Us) of the VZV genome [14, 28, 29].

Our initial studies included the cloning of VZV SalI-I DNA and the expression of VZV gI and gE using an in vitro transcription-translation system (Fig. 2). Antibodies were generated in rabbit against the in vitro translation (ivt) products encoded by VZV gI and gE genes and reacted with VZV-infected cells. The results demonstrated that native VZV gI and gE were recognized by antibodies induced against ivt products (Fig. 2). These results further substantiated the location of VZV gE open reading frame on the VZV genome.

Expression of VZV gE by vaccinia virus

VZV gE (gpI) gene was inserted into the vaccinia genome by homologous recombination [33] and the recombinant viruses expressing gE (RVVgE) were analyzed by monoclonal antibodies directed against VZV gE (Figs. 3, 4, and 5). The results indicated the expression of VZV gE by RVVgE with a similar size to native gE expressed in the infected cells [34]. In addition, recombinant vaccinia virus expressing VZV gE were capable of inducing VZV-neutralizing antibodies [35]. These results indicated that RVVgE has the potential application as a VZV subunit vaccine. However, since the use of recombinant vaccina viruses as subunit vaccines had not been approved by the regulatory agencies, our efforts were concentrated on the construction of a secretory VZV gE which can be purified from tissue culture fluids of the infected cells and used as a VZV subunit antigen.

Expression of secretory truncated VZV gE

VZV gE gene was cleaved with various restriction endonucleases to generate truncated gE DNA fragments, encoding the N-terminal region of gE with 124, 160, 316 and 511 amino acid residues, respectively (Fig. 6). Recombinant vaccinia viruses expressing truncated VZV gE DNA fragments (VVTgE-124, VVTgE-160, VVTgE-316, VVTgE-511) were then constructed (Fig. 9). Our initial studies on truncated VZV gE proteins were focused on a recombinant vaccinia virus expressing the truncated gE with 160 amino acid residues (VVTgE-160). Analysis of VVTgE-160-infected cells indicated the

Table 1: Varicella-Zoster Virus Infection

Primary Infection -- Latency -- Reactivation in immunosuppressed and elderly (dorsal root ganglia)

Varicella (chickenpox)	Zoster (shingles)
Complications:	Complications:
Congenital malformations	Postherpetic neuralgia
Myocarditis	Encephalitis
Encephalitis	Pneumonitis
Delayed Hemiplegia	Myelitis
Visceral dissemination	Cerebral infarction
Cerebral vasculopathy	Disseminated zoster
Persistent skin lesions (in AIDS patients)	Also associated with: Systemic lupus erythematosus Neurologic disease without skin rash

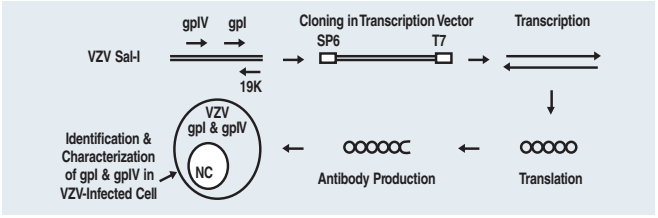

Fig. 1. Cloning, expression of VZV SalI-I DNA fragments in an in vitro transcription system and generation of antibodies in rabbit against the in vitro translation products which were used to identify native VZV gI and gE in the infected cells [30, 31].

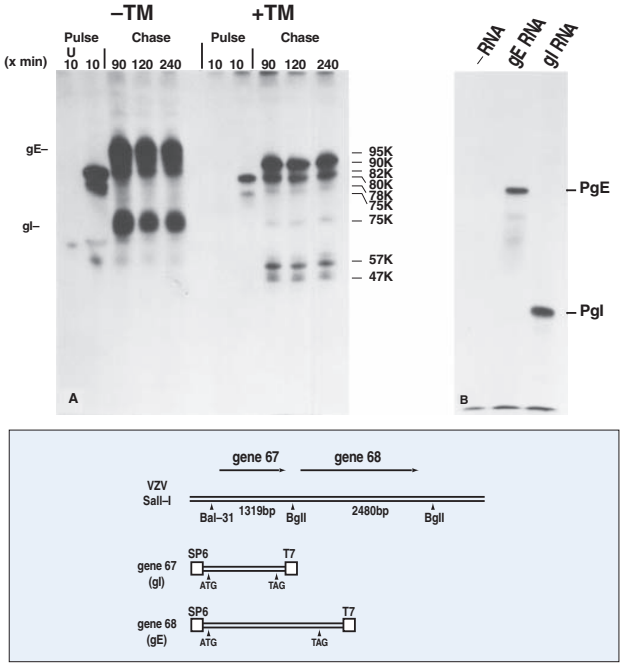

Fig. 2. Analysis of VZV gI and gE with antibodies raised in rabbit against the in vitro translation (ivt) products encoded by VZV genes 67 and 68. VZV SalI-I DNA fragment containing genes 67 and 68 was cloned into pGEM transcription vector. RNA transcribed from one strand was translated in vitro and antibodies were raised in rabbit against the ivt products (RAnti-gI & gE) and used to identify and characterize VZV gI and gE in the infected cells. (A) pulse-

chase experiments of VZV-infected cells immunoprecipitated with RAnti-gI & gE. Infected cells were labeled with [35S]methionine for 10 min in the absence (-) or presence (+) of tunicamycin (TM), which inhibits the addition of N-linked oligosaccharides to the native protein [32]. After being pulse-labeled, cells were either harvested or the label was chased for 90, 120 and 240 min. Infected cell lysates were immunoprecipitated with RAnti-gI and gE and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). U, uninfected cells were pulse-labeled for 10 min. (B) SDS-PAGE Analysis of proteins encoded by genes 67 and 68. The predicted open reading frame (ORF) of genes 67 and 68 was cloned into pGEM vector, RNA was transcribed from each ORF, translated in vitro and the products were analyzed by SDS-PAGE. The size of the precursor-products of gI and gE in the absence or presence of TM are indicated. Lane 1 in B (-RNA), no RNA was included in the translation reaction. pgI and pgE represent the precursor proteins to VZV gI and gE.

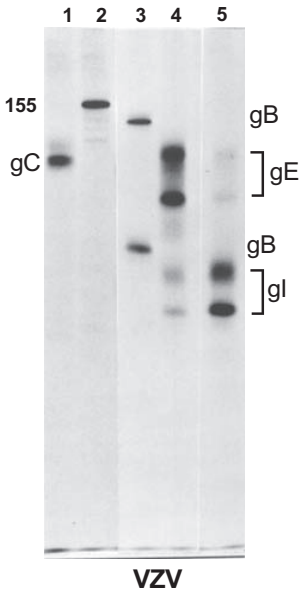

Fig. 4. Immunoprecipitation of VZV glyco-proteins. VZV-infected cells were labeled with [35S]methionine and cell lysates were reacted with monoclonal antibodies directed against VZV gE, VZV gB, VZV gH, VZV gI and VZV nucleocapsid protein with a size of 155 kDa [13].

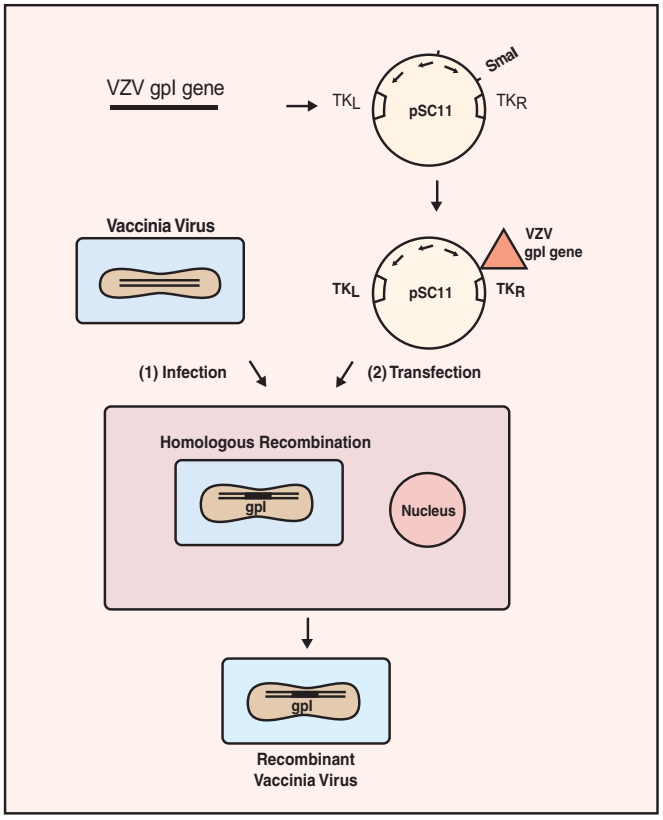

Fig. 3. Construction of recombinant vaccinia virus expressing VZV gE. VZV gE gene was cloned in pSC11 insertion vector. Cells (tk$^-$ cells) were first infected with wild-type vaccinia virus and then were transfected with recombinant insertion vector. Recombinant vaccinia viruses carrying VZV gE were selected and analyzed for the expression of VZV gE.

expression of a truncated gE which was recognized by gE-specific monoclonal antibodies (Fig. 7). In addition, antibodies raised in rabbit (RAnti-VVTgE-160) reacted with the native gE in VZV-infected cells (Fig. 8). Furthermore, neutralization tests showed that RAnti-TgE-160 neutralized VZV infectivity. However, the neutralization activity by RAnti-TgE-160 was 50% less than that observed with antibodies raised against recombinant vaccinia virus expressing the entire VZV gE (RVVgE).

To enhance the antigenicity of the truncated VZV gE, a truncated gE DNA fragment encoding the N-terminal region of gpI with 511 amino acid residues was expressed by vaccinia virus (VVTgE-511) (Fig. 9). The expression of VVTgE-511 in the infected cells and in tissue culture fluids (TCF) was analyzed by pulse-chase experiments (Fig. 10). The results showed the expression of a polypeptide with an apparent size of 60kDa by VVTgE-511 which was processed to a mature form of 76kDa protein [36]. The results also demonstrated that the mature form of truncated VZV gE with 511 amino acids (previously designated TgpI-511) was secreted from VVTgE-511-infected cells and was recognized by VZV gE-specific monoclonal antibodies (Fig. 10). These results indicated that the anchoring region of VZVgE

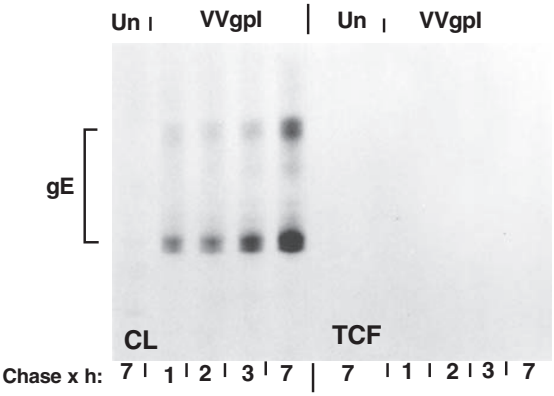

Fig. 5. Expression of VZV gE by recombinant vaccinia virus (RVVgE). Cells were infected with RVVgE, pulse-labeled for 30 min with [35S]methionine and chased for 1, 2, 3 and 7 hours. Cell lysates (CL) and tissue culture fluids (TCF) were immunoprecipitated with VZV gE-specific monoclonal antibodies and analyzed by SDS-PAGE. Un, uninfected cells were pulse-labeled for 30 min and chased for 7 hours.

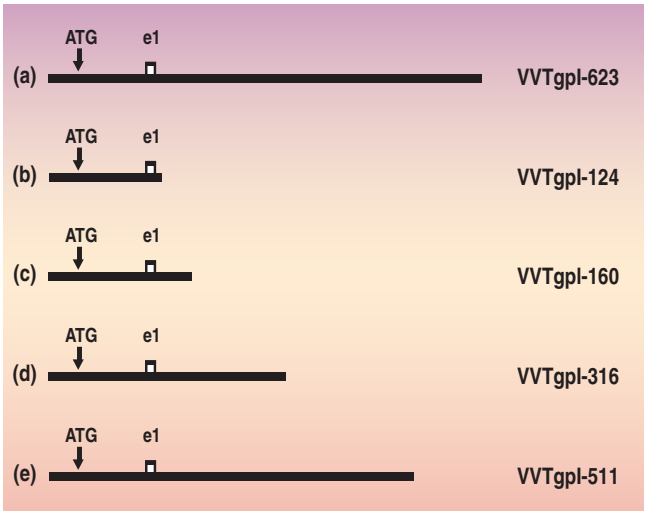

Fig. 6. Recombinant vaccinia viruses expressng VZV gE with 124 (VVTgE-124), 160 (VVTgE-160), 316 (VVTgE-316) and 511 (VVTgE-511) amino acid residues were constructed by truncation of gE gene and inserted into the vaccinia virus genome. (a) represents the entire coding region of VZV gE with 623 amino acid residues, expressed by vaccinia virus (RVVgE-623). Locations of VZV gE initiation codon (ATG) and e1 epitope are indicated.

is located near or on the C-terminus of this glycoprotein.

Immunogenicity of truncated VZV gE (TgE-511)

To examine the antigenicity of TgE-511 (TgpI-511), rabbits were immunized with purified preparation of VZV TgE-511 (Fig. 11, lane 1). The serum samples from immunized animals were analyzed by immunoprecipation. The results showed that purified TgE-511 was capable of inducing antibody responses which were reactive with TgE-511 (Fig. 11, lane 2).

To determine virus neutralizing activity of anti-TgE-511 antibodies, neutralization tests were performed in the presence or absence of complement. The results revealed plaque reduction in VZV-infected cells of more than 80% with 1:10 and 1:100 dilutions of antibodies raised against VVTgE-511 and purified TgE-511, respectively, in the presence of complement (Table 2). The neutralization tests demonstrated that purified TgE-511 is capable of inducing complement dependent neutralizing antibodies. These results suggested that the purified preparation of TgE-511 may have the potential to be used as a candidate VZV subunit vaccine.

Antibody-binding sites (epitopes) on VZV TgE-511 antigen

The immunogenicity of TgE-511 in animals provided the rationale for further testing in human clinical trials to determine whether this glycoprotein can stimulate immune responses in humans. However, since truncation may have resulted in conformational changes of the native VZV gE, before any clinical trials it was necessary to determine whether conformation-independent epitopes were present on TgE-511 glycoprotein and whether these epitopes were reactive with anti-gE antibodies in human sera.

To determine whether VZV TgE-511 is recognized by human anti-gE-specific antibodies, purified TgE-511 was immunoprecipitated with serum samples from individuals with no clinical symptoms of VZV infection but with a history of childhood chickenpox. The results showed that VZV seropositive human serum samples were reactive with TgE-511 subunit vaccine (Fig. 12).

To identify the location of antibody-binding sites (epitopes) on TgE-511, three different forms of secretory truncated VZV gE proteins were expressed by recombinant vaccinia viruses (Fig. 6). These truncated gE (designated TgE-124, TgE-160 and TgE-316) as well as TgE-511 were immunoprecipitated with VZV-seropositive human sera and VZV gE-specific monoclonal antibodies and analyzed by SDS-PAGE (Fig. 13). The results from these studies supported our previous findings regarding the location of VZV gE e1 epitope and identified an additional epitope reactive with monoclonal antibody G7 (MAbG7) within the same region of gE (Fig. 18). In addition, the results provided evidence for the existence of three additional epitopes within amino acid sequences 124 to 511 of VZV gE (Figs. 13 and 18).

The glycosylation sites (N-linked and O-linked) on TgE-511 were also determined by tunicamycin treatment and O-glycanase enzyme digestion. Secondary structure prediction of VZV gE with 623 amino acid residues has identified the location of four putative N-linked glycosylation sites on gpI [37], three of which are located within the central region between residues 265 to 440 and one site near the carboxy-terminal region of gE (Fig. 14). Our studies with tunicamycin treatment and O-glycanase digestion supported the previous findings and indicated the existence of N-linked and O-linked glycosylation sites on the truncated VZV glycoprotein with 511 amino acid residues (Figs. 15 and 16). The results showed the presence of N-linked glyco-sylation sites within amino acid sequences 160-511 of TgE-511 (Fig. 17). The location of antibody-binding sites (epitopes) and glycosylation sites on TgE-511 are shown in Fig. 17.

The results from these studies revealed the presence of both N-linked and O-linked oligosaccharides on TgE-511. In addition, these results identified the location of new epitopes on VZV truncated gE and demonstrated that the epitopes on this glycoprotein were recognized by human sera from VZV-seropositive individuals. The immunogenicity of TgE-511 glycoprotein antigen in animals, along with its reactivity with VZV-specific antibodies in human sera suggested that TgE-511 may be considered as a VZV subunit vaccine.

Boosting human immune response with candidate VZV subunit glycoprotein vaccine (TgE-511)

The immunogenicity of TgE-511 subunit vaccine in animals and its reactivity with human sera provided the rationale for testing the immunogenicity of this protein in human. A VZV seropositive individual was immunized with 100 μg of purified VZV TgE-511 glycoprotein subunit antigen [38]. Serum samples were obtained during a 40-day period post-immunization (PI) and analyzed by immunoprecipitation and virus neutralization tests. The results from immunoprecipitation studies revealed an increase in VZV anti-gE antibody titer as early as 6 days PI which continued to rise during a period of 40 days PI (Fig. 18, Table 3).

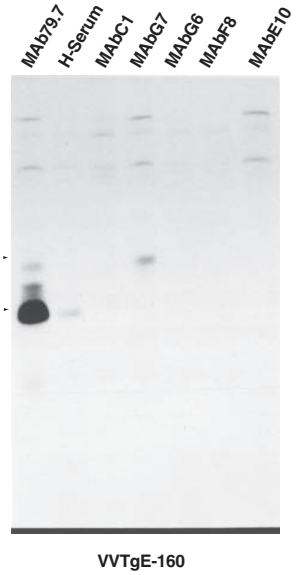

Fig. 7. Expression of a truncated VZV gE (TgE-160) by recombinant vaccinia virus. Cells were infected with RVVTgE-160, labeled with [35S]methionine, cell lysates were immunoprecipitated with monoclonal antibodies (MAb) which are recognized by VZV gE e1 epitope (MAb79.7, MAbG7), VZV seropositive human serum (H-Serum) and MAbs which are directed against other VZV gE epitopes (MAbC1) or other VZV glycoproteins (MAbG6, MAbF8, MAbE10), and analyzed by SDS-PAGE. The apparent size of precursor-products of TgE-160 are indicated.

Expression and immune response to a non-glycosylated VZV TgE-511 (nTgE-511)

To determine whether non-glycosylated VZV TgE-511 is capable of inducing neutralizing antibodies, the truncated gE gene encoding 511 amino acid residues was cloned and expressed in three different prokaryotic expression vectors (pMal, TrxHis, TrxFus). The results from these studies revealed a low yield of nTgE-511 in all three expression systems. This could be due to the lack of glycosylation machinery in prokaryotic cells (*E. coli*) which is required for the processing of VZV TgE-511 glycoprotein. In addition, antibodies raised in rabbits against nTgE-511 showed neutralization activity approximately 70% less than that obtained from antibodies raised against fully glycosylated TgE-511. These results suggested that: (1) prokaryotic expression systems (e.g., *E. coli*) may not be suitable for the production of large quantities of TgE-511 protein; and (2) glycosylation of TgE-511 may be required for the induction of neutralizing antibodies in animals as well as in humans.

In vitro stimulation of human mononuclear cells with VZV TgE-511 subunit vaccine

Mononuclear cells (MNC) from VZV-seropositive individuals were separated and stimulated with either purified VZV TgE-511 glycoprotein or purified VZV glycoproteins mix. Tissue culture fluids from the stimulated MNC were then analyzed for the presence of VZV gE-specific antibodies using indirect immunofluorescent tests. The results indicated that TgE-511 was capable of *In Vitro* stimulation of MNC and induction of VZV gE-specific antibodies (Table 4). These results demonstrated that MNC from individuals with a past history of childhood chickenpox (varicella) recognized purified TgE-511 glycoprotein subunit vaccine.

Stability of gE Epitopes

The epitope stability of VZVgE was analyzed with monoclonal antibodies (MAbs) in cells infected with different passages of five various VZV strains and seventeen different isolates from varicella and zoster patients [39, 40]. The gE-specific MAbs recognized same antigenic sites (epitopes) in VZV isolates with various passage history. All VZV strains and virus isolates reacted with anti-gE monoclonal antibodies by immunoprecipitation, or indirect fluorescent antibody staining test. In addition, sera from VZV seropositive individuals reacted with VZV subunit vaccine (TgE-511). Also, human mononuclear cells (MNCs) stimulated with TgE-511 glycoprotein were shown to produce VZV-specific antibodies *in vitro*.

The stability of VZVgE epitopes was further analyzed by sequencing the gE gene encompassing two epitopes (e1 and c1) to determine any variation among 32 VZV isolates from different geographical locations. Eleven isolates showed variance when compared with the sequenced Dumas VZV strain [14] through base substitutions, with two isolates showing an amino acid change of tryptophan to arginine outside the coding regions of the epitopes recognized by monoclonal antibodies C1 and 4F9.

These results demonstrated the stability of gE epitopes in TgE-511 and among the VZV-isolates obtained from different passages, suggesting that VZV gE with conserved epitopes could be used as therapeutic booster vaccines in adults and the elderly to prevent zoster.

Conclusions

Our studies have shown that a truncated form of VZV glycoprotein E (TgE-511) antigen: (1) contains conserved epitopes; (2) elicits the induction of neutralizing antibodies in animals as well as in humans; and (3) stimulates the induction of VZV gE-specific antibodies in VZV-seropositive human mononuclear cells. Such a VZV glycoprotein antigen, therefore, may have the potential to be used as a candidate VZV glycoprotein subunit vaccine for the prevention of primary VZV infection (varicella) or boosting of immune response against VZV reactivation (zoster) in adults, the elderly and immunosuppressed individuals.

It is known that both VZV-specific cell-mediated immunity (CMI) and neutralizing antibody are crucial in immunity to VZV infection, and waning CMI plays a role in the reactivation of VZV (zoster). Due to the immune senescence, the specific immune responses for VZV antigens may decline with age and their capacity to prevent herpes zoster (shingles) will diminish. This will result in an increased reactivation of zoster in individuals over the age of 45. Therefore, augmentation of immunity to VZV may reduce the likelihood of virus reactivation (zoster) in the elderly. It has been shown that live attenuated, as well as killed VZV vaccine are capable of stimulating immune responses in adults and the elderly and thereby may prevent herpes zoster [41-44]. However, since both killed and live VZV vaccine contain viral DNA and may develop latency and reactivate to produce zoster, and that the live VZV vaccine may have adverse effects in immunocompromised patients, there is a need for a safer VZV booster vaccine that will stimulate protective immune responses without establishing viral latency. Our studies have shown the stimulation of VZV neutralizing antibodies in a VZV-seropositive human subject by TgE-511 subunit vaccine, suggesting that this glycoprotein can be used as a booster vaccine in adult VZV seropositive population. The immunogenicity of TgE-511 subunit vaccine in humans provides the rationale for further testing in human clinical trials to determine whether such a subunit vaccine can prevent or reduce virus reactivation in the elderly and immunosuppressed individuals who are at increased risk of developing herpes zoster. In addition, since there is no test to identify high-risk patients for developing zoster, all adults over the age of 45 may benefit from a booster subunit vaccine to reduce the risk of VZV reactivation.

Acknowledgements

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Table 2: Varicella-zoster virus neutralization test[a]

		Number of plaques[b] (with complement)			Plaque reduction (%)			Number of plaques (without complement)			Plaque reduction (%)		
Antibody	Target protein	1:10	1:100	1:1000	1:10	1:100	1:1000	1:10	1:100	1:1000	1:10	1:100	1:1000
RAnti-VVgE[c]	VZVgE	6.0	8.0	30.5	91.9	90.2	62.8	52.0	55.0	46.0	8.8	10.6	28.1
RAnti-VVgI[d]	VZVgI	5.5	7.0	23.0	92.6	91.5	71.9	45.5	51.5	56.0	20.2	16.3	12.5
RAnti-VVTgE-511[e]	VZVTgE-511	7.5	9.5	29.0	89.9	88.4	64.6	52.0	55.0	57.5	8.8	10.6	10.1
RAnti-pTgE-511[f]	VZVTgE	7.0	9.0	24.0	90.6	89.0	70.7	45.0	48.0	52.0	21.0	21.9	18.7
NRS[g]	—	78.5	80.0	87.0	—	2.4	—	54.0	56.0	63.5	5.3	8.9	0.8
—	—	74.5	82.0	82.0	—	—	—	57.0	61.5	64.0	—	—	—

[a]Neutralization tests were performed by the constant-varying serum technique (dilutions: 1:10; 1:100; 1:1000) in the absence or presence of 0.2 ml guinea-pig complement (Cappel Research Products) per reaction
[b]Average number of plaques in duplicate wells of six-well plates, determined with 1:10, 1:100 and 1:1000 dilutions of each serum
[c]RAnti-VVgE, rabbit antibodies against recombinant vaccinia virus expressing VZV gE
[d]RAnti-VVgI, rabbit antibodies against recombinant vaccinia virus expressing VZV gpI
[e]RAnti-VVgE-511, rabbit antibodies against recombinant vaccinia virus expressing a truncated VZVgE with 511 amino acid residues
[f]RAnti-pTgE-511, rabbit antibodies against purified truncated gpl with 511 amino acid residues
[g]NRS, normal rabbit serum obtained from a non-immunized rabbit

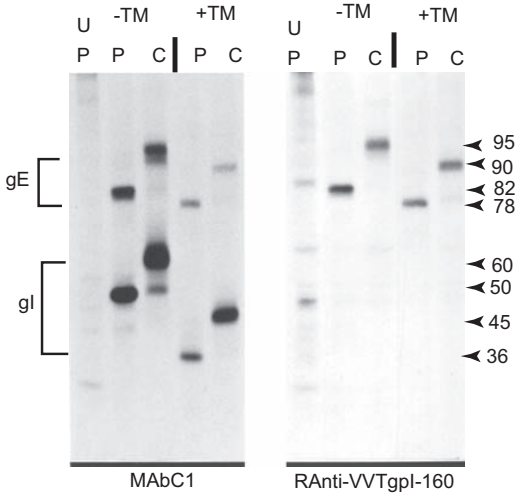


Fig. 8. Pulse-chase experiments and immunoprecipitation of VZV-infected cells with RAnti-VVTgE-160. (A and B) cells were infected with RVVTgE-160 and pulse-labeled (P) with [35S] methionine for 10 min in the absence (-) or presence (+) of tunicamycin (TM) which inhibits the addition of N-linked polysaccharides to the native protein. Cells were either harvested or the label was chased (C) for 60 min. Cell lysates were prepared and immunoprecipitated with either a monoclonal antibody (MAbC1) which reacts with both VZVgE and gI or RAnti-VVTgI-160. Samples were analyzed by a 10% SDS-PAGE. U, uninfected cells were pulse-labeled (P) for 10 min. Apparent sizes (kilodaltons) of the precursor-products of VZV gI and gE which are recognized by MAbC1 and RAnti-VVTgE-160 are indicated.

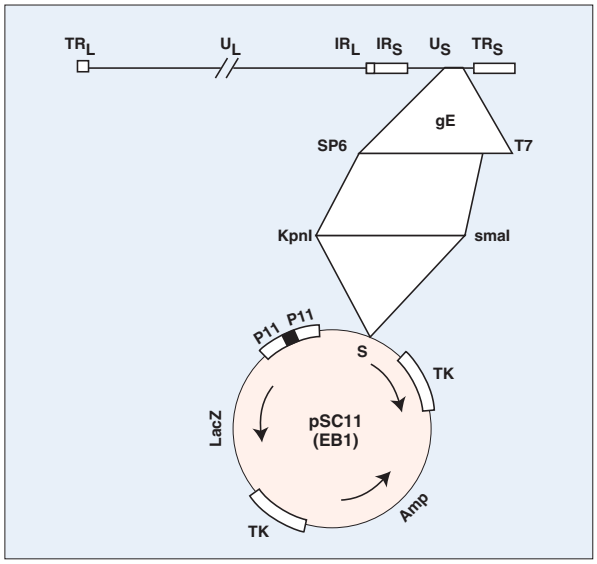


Fig. 9. Construction of a recombinant vaccinia virus insertion vector carrying a truncated VZV gE gene. A 2.5 kb BglI DNA fragment (containing VZV gE open reading frame) was cleaved with kpnI and XmaIII, electroeluted, blunt-ended and cloned at the SmaI (S) site of vaccinia virus insertion vector pSC11 (EB1). The truncated VZV gE DNA fragment (1640 bp) encodes the N-terminal region of gE with 511 amino acid residues. A recombinant vaccinia virus expressing the truncated gE (RVVTgE-511) was constructed as described in Fig. 11. A physical map of VZV DNA and the location of gE on the viral genome are shown.

Table 3. Varicella Zoster Virus Neutralization Test[1]

Antibody	Number of plaques[2]	Plaque Reduction (%)
1. Pre-immunization (ODPI)[3]	75.0	16.5
2. 6 DPI	71.0	21.0
3. 15 DPI	25.0	72.2
4. 33 DPI	15.5	82.7
5. 40 DPI	3.0	96.7
6. VZV Seronegative (VZV^-)[4]	86.0	4.3
7. VZV Seropositive (VZV^+)[5]	2.0	97.8
8. No antibody	89.9	—

[1]Neutralization tests were performed by the constant-varying serum technique (dilution 1:100) in the presence of 0.2 ml guinea- pig complement (Cappel Research Products).
[2]Average number of plaques in duplicate wells of six-well plates, determined with 1:100 dilution of each serum.
[3]DPI, days post immunization
[4]Serum sample from a VZV seronegative (VZV^-) individual
[5]Serum sample from a zoster (VZV^+) patient obtained four weeks after the appearance of skin rash.

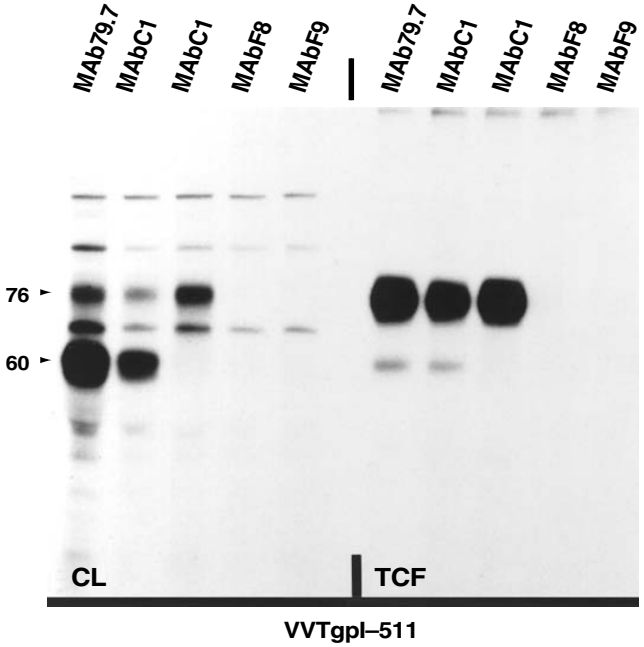


Fig. 10. Expression of the truncated VZV gE (TgE-511) by recombinant vaccinia virus (VVTgE-511). Cells were infected with VVTgE-511 and pulse-labeled with [35S]methionine for 60 min. Infected cells were either harvested or washed and the label was chased for 2 hr. Cell lysates (CL) from pulse-labeled experiments and tissue culture fluids (TCF) from cells which had been pulse-chased were immunoprecipitated with gE-specific monoclonal antibodies (MAb79.7, MAbC1, MAbG7) and MAbF8 and MAbF9 which are directed against VZV gB and a 155 kDa VZV nucleocapsid protein, respectively. The samples were analyzed by 10% SDS-PAGE. The size (kDa) of precursor and processed form of TgE-511 are shown on the left.

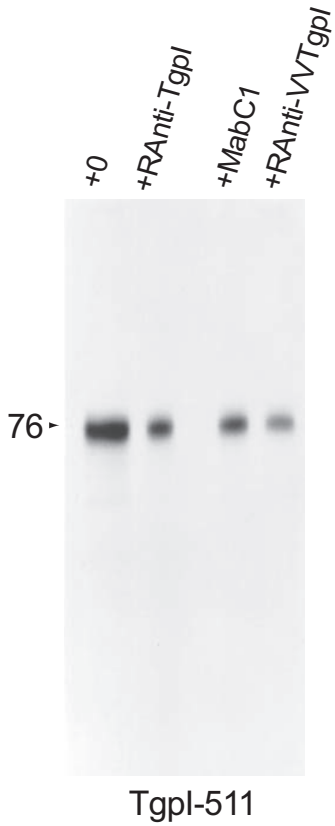


Fig. 11. Immunoprecipitation of purified truncated VZV gE (TgpI-511). TgE-511 was purified (lane 1) from tissue culture fluids of VVTgE-511-infected cells. Antibodies were raised in rabbit (RAnti-TgE) against TgE-511 and used to immunoprecipitate purified TgE-511 (lane 2). Purified TgE-511 was also immunoprecipitated with VZV gE-specific monoclonal antibody (MAbC1) (lane 3) and antibodies prepared in rabbit (RAnti-VVTgE) against recombinant vaccinia virus expressing TgE-511 (lane 4). The samples were analyzed by 9% SDS-PAGE. The size (kDa) of the mature form of TgE-511 is indicated on the left.

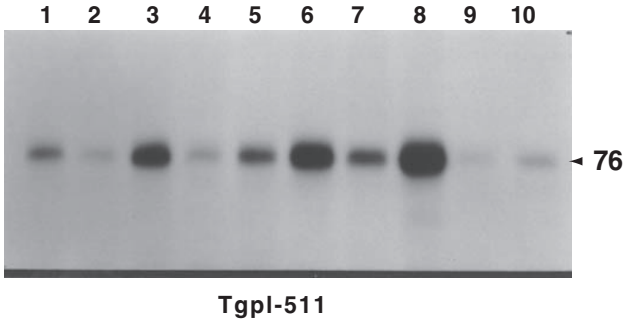


Fig. 12. Immunoprecipitation of purified VZV TgE-511 glycoprotein with human sera. Cells were infected with recombinant vaccinia virus expressing TgE-511, labeled with [35S]menthionine and labeled TgE-511 was purified from tissue culture fluids [36]. The radioactively labeled purified TgE-511 was immunoprecipitated with human sera (lanes 1-10) and analyzed by 10% SDS-PAGE. The apparent size (kDa) of the TgE-511 is shown on the right.

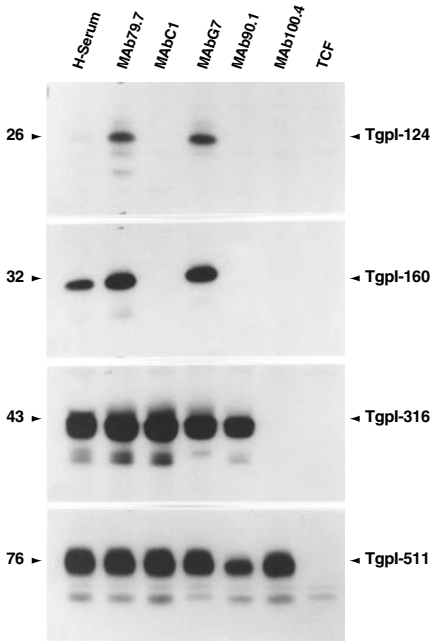


Fig. 13. Localization of antibody-binding sites (epitopes) on truncated VZV TgE-511 glycoprotein. Recombinant vaccinia viruses expressing truncated VZV gE with amino acid residues of 124 (VVTgE-124), 160 (VVTgE-160), 316 (VVTgE-316) and 511 (VVTgE-511) were constructed. Cells were infected with recombinant vaccinia viruses, labeled with [35S] methionine and the secretory truncated gE (TgE-124, TgE-160, TgE-316 and TgE-511) were immunoprecipitated with VZV-seropositive human serum (H-serum), gE-specific monoclonal antibodies (MAb79.7, MAbC1, MAbG7, MAb90.1 and MAb100.4) and RPMI-1640 tissue culture fluid (TCF). The samples were analyzed by 10-12% SDS-PAGE. Apparent sizes (kDa) of the secretory truncated VZV gE are shown on the left.

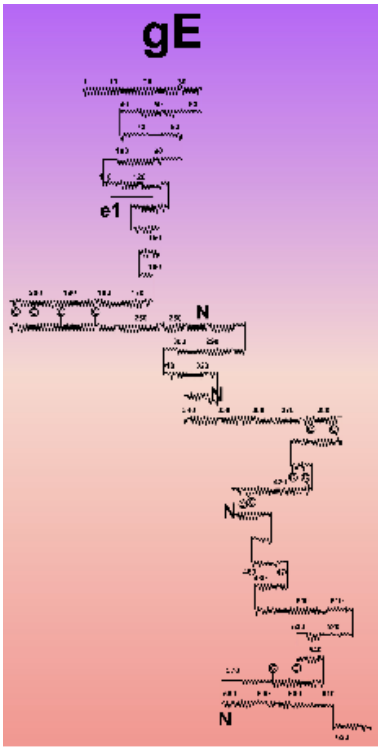


Fig. 14. Secondary structure prediction of VZV gE derived from VZV DNA sequences [14]. N, indicates the putative N-linked glycosylation sites. Open circles refer to cystein residues on gE; e1 indicates the antigenic epitope on VZV gE.

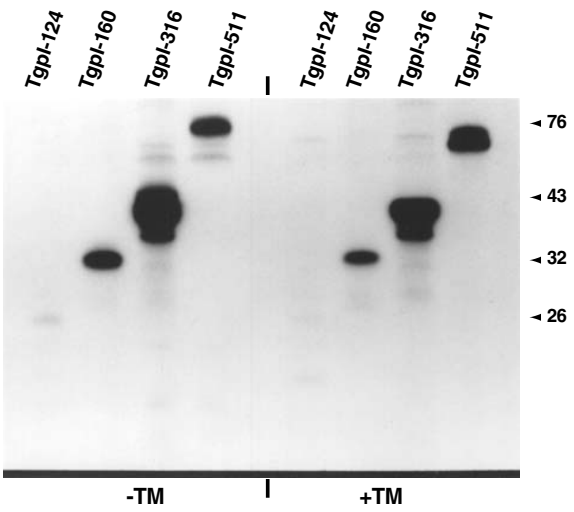


Fig. 15. N-linked glycosylation of truncated VZV gE (TgE-124, TgE-160, TgE-316, TgE-511). Cells were infected with recombinant vaccinia viruses (VVTgE-124, VVTgE-160, VVTgE-316, VVTgE-511), pulse-labeled with [35S]methionine for 60 min in the absence (-) or presence (+) of tunicamycin (TM). Cells were washed and the label was chased for 120 min. Tissue culture fluids (TCF) were harvested, immunoprecipitated with VZV gE-specific monoclonal antibody (MAb79.7) and analyzed by 12% SDS-PAGE [45]. The sizes (kDa) of the mature products of each truncated VZV gE are shown on the right.

Table 4. *In Vitro* Stimulation of Human Mononuclear Cells (MNC) with VZV TgE-511 Antigen*

Patient			Fluorescent Antibody (FA) Test	
Number	Sex	Age	TgE-511	VZVgps
1	Female	33	+	+
2	Female	46	+	+
3	Female	58	+	+
4	Female	49	+	+
5	Female	53	+	+
6	Female	81	+	+
7	Female	44	+	+
8	Female	80	+	+
9	Female	51	+	+
10	Male	77	+	+

*Blood samples from ten patients with a past history of childhood chickenpox (varicella) were collected, mononuclear cells (MNC) were separated and each sample was stimulated with 3μg of either purified VZV TgE-511 glycoprotein antigen or purified vzv glycoprotein mix containing VZV gE, gB, gH and gI. MNC samples were incubated at 37½C for 72 hours in RPMI-1640 media containing 10% fetal bovine serum (FBS). Tissue culture fluids (TCF) were collected and examined for anti-gE antibodies by fluorescent antibody (FA) test.

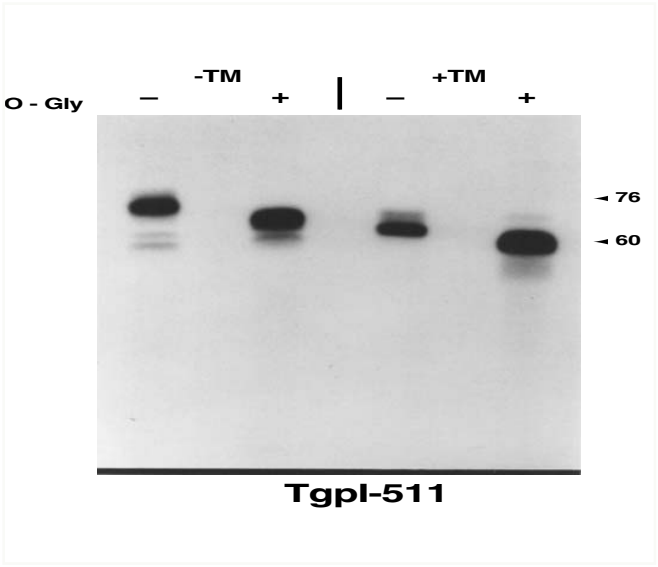


Fig. 16. Demonstration of N-linked and O-linked oligosaccharides on truncated VZV gE (TgE-511) subunit vaccine. Cells were infected with VVTgE-511, pulse-labeled with [35S]methionine for 60 min in the absence (-) or presence (+) of tunicamycin (TM). Cells were washed and the label was chased for 120 min [45]. Tissue culture fluids (TCF) were harvested, immunoprecipitated with VZV gE-specific monoclonal antibody (MAb79.7) and analyzed by 10% SDS-PAGE (lanes 1 and 3). TCF were also reacted with MAb79.7 and the immune compleses were precipitated with S. aureus, washed and treated with neuraminidase followed by O-glycanase. The samples were analyzed by 10% SDS-PAGE (lanes 2 and 4). The sizes (kDa) of the precursor and mature form of the truncated VZV gpI (TgE-511) are indicated.

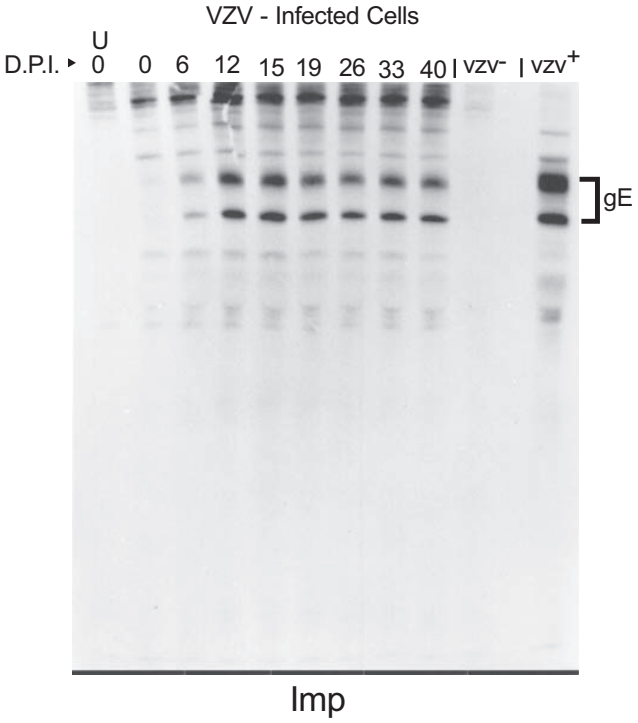
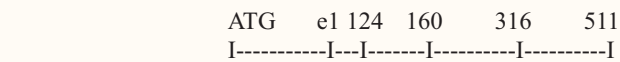


Fig. 17. Immunoprecipitation of VZV-infected cell lysates with human sera obtained from an individual immunized with purified VZV TgE-511 subunit antigen. VZV-infected cells were labeled with [35S]methionine and cell lysates were immunoprecipitated with sera obtained from the immunized individual at 0, 6, 12, 15, 19, 26, 33 and 40 days post-immunization (D.P.I.). U, uninfected cell lysates were immunoprecipitated with serum samples obtained at 0 D.P.I. Infected cell lysates were immunoprecipitated with a serum sample from a VZV-seronegative (VZV^-) patient and with a serum sample from a zoster (VZV^+) patient. The serum sample from the zoster patient was obtained 4 weeks after the appearance of skin rash. Samples were analyzed by 10% SDS-PAGE. The location of the precursor-products of VZV gE is indicated on the right.

A.



B.

Recombinant Vaccinia Virus	Human Sera	MAb 79.7	MAb C1	MAb G7	MAb 90.1	MAb 100.4	N-Gly
VVTgE-124	±	+	-	+	-	-	-
VVTgE-160	+	+	-	+	-	-	-
VVTpE-316	+	+	+	+	+	-	+
VVTgE-511	+	+	+	+	+	+	+

Fig. 18. Location of conformation-independent epitopes and glycosylation sites on VZV TgE-511 subunit vaccine. (A) Map locations (at amino acid residues 124, 160, 316, and 511) of truncated VZV gE DNA fragments expressed in vaccinia virus. Location of initiation codon (ATG) and VZV e1 epitope on the truncated VZV gE are indicated. (B) Recombinant vaccinia viruses (VVTgE-124, VVTgE-160, VVTgE-316, VVTgE-511) were prepared by cloning four truncated gE fragments in pSC11 (EB1) plasmid vector and insertion into vaccinia virus. Expression of recombinant vaccinia viruses were analyzed by human sera and VZV gE-specific monoclonal antibodies. The N-linked glycosylation (N-Gly) sites on truncated VZV gE were determined by treatment of truncated gE with tunicamycin and O-glycanase digestion as shown.

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