Identification of antigenic epitopes for Guertu virus nucleocapsid protein

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Abstract

Guertu virus (GTV), a novel tick-borne virus with potential pathogenicity, was first isolated from Dermacentor nuttalli in Xinjiang, China, in 2014. GTV has been shown to infect animal and human cell lines and to be pathogenic in mice. The viral nucleoprotein (NP) is the most conserved immunogenic protein. Elucidating the B-cell epitopes (BCEs) in the immunodominant region of the NP is important for the development of virus detection methods and vaccines. In order to identify the minimal motifs of linear BCEs in the NP of the GTV DXM strain, we used an improved biosynthetic peptide (BSP) method to truncate GTV NP into 30 16mer-peptides with 8 overlapping amino acid residues spanning the full length of the protein. The peptides were analyzed by western blot using rabbit anti-GTV NP polyclonal antiserum, and four positive 16mer-peptides were obtained. The 16mer-peptides were then truncated into 31 8mer-peptides with 7 overlapping amino acid residues and 10mer-peptides with 9 overlapping amino acid residues to screen for BCEs that can react with the rabbit anti-GTV NP polyclonal antiserum. The results showed that there were 6 minimal BCE motifs, namely, Enp1, ^{88}EKYGLVER^{95}; Enp2, ^{88}EKYGLVER^{95}; Enp3, ^{162}TTKILMEA^{169}; Enp4, ^{187}GASKAEVY^{194}; Enp5, ^{191}AEVYNSFR^{198}; and Enp6, ^{236}ETAAAAYRNL^{245}. Positive sheep sera could recognize all six BCEs with anti-GTV antibodies. The BCEs were aligned with the sequences of eight representative severe fever with thrombocytopenia syndrome phlebovirus strains from different countries and regions that were evolutionarily closely related to GTV. The sequence identity of the BCEs ranged from 80–100%, thus showing high conservation. The fine epitope mapping of GTV NP can be used to explore the biological and immunological properties of GTV NP antigens and serve as basic data for the development of multi-epitope detection reagents and vaccine design for GTV.

Introduction

Tick-borne viruses (TBVs) can cause natural-focal diseases using ticks as vectors. By capitalizing on the superior adaptability of ticks and their viral preservation characteristics and transmission, TBVs can circulate and survive in nature for a long time[1]. New TBVs have emerged continuously in recent years, causing substantial losses to people's lives in epidemic foci. However, effective vaccines and therapeutic antibodies are currently unavailable for most TBVs, especially for novel TBVs[2]. Banyangvirus is an emerging TBV belonging to the family Phenuiviridae of the order Bunyavirales[3,4]. The latest classification by the International Committee on Taxonomy of Viruses (ICTV) has clarified that members of this genus include severe fever with thrombocytopenia syndrome phlebovirus (SFTSV), heartland virus (HRTV), and Guertu virus (GTV) [3].

GTV was first isolated in 2014 from Dermacentor nuttalli around the Tianshan mountainous region in the southern part of Wusu, Xinjiang, China[5]. In vivo and in vitro studies of GTV have shown that it can effectively infect human and animal cell lines and has characteristics of pathological damage similar to those caused by SFTSV in infected mice[5–7]. Furthermore, antibodies with neutralizing activity against

GTV were identified in serum samples from residents living in the Wusu region of northern Xinjiang[5], indicating that this virus is a potential pathogen that poses a threat to animals and humans.

As with other members of the genus Banyangvirus, GTV is a negative-strand RNA virus with three segments of negative-strand RNA genes, consisting of the large (L), medium (M), and small (S) segments. The L and M segments encode RNA-dependent polymerase and envelope glycoproteins (Gn and Gc), respectively, while the S segment encodes the nucleocapsid protein (NP) and non-structural proteins (NSs). NP binds to and protects the virus genomic RNA and interacts with the RNA-dependent RNA polymerase to form the core-NP complex for viral transcription and replication[8,9]. In addition to its crucial role in protecting viral RNA, NP is also involved in RNA transcription and replication and virion assembly. Furthermore, it is the most conserved structural protein in the family Bunyavirales[10,11]. This led us to believe that NP can potentially be used in GTV assays and epitope vaccines.

Gene sequencing analysis showed that GTV has up to 90% sequence identity with SFTSV and 86% amino acid sequence identity for NP, replacing SFTSV NP to maintain self-replication[5]. Thus, we speculate that the GTV NP, as with the SFTSV NP, may also be capable of inducing a large number of specific antibody responses in patients infected with GTV[12]. Therefore, elucidating the B-cell epitopes (BCEs) in conserved or immunodominant regions of GTV-NP is essential for the development of multi-epitope antigen-based viral assays and vaccines.

In this study, we chose an improved overlapping biosynthetic peptide (BSP) strategy[13] to identify the linear BCEs of GTV-NP. Based on the NP amino acid sequence of the GTV DXM strain (GenBank accession number: KT328591.1), GTV-NP was divided into 16mer-peptides with 8 overlapping amino acid residues covering the full length of the protein and into 8-mer peptides with 7 overlapping amino acid resides. The peptides were inserted into the prokaryotic expression vector, pXXGST-3, making the BSP strategy more straightforward and easier to implement. pXXGST-3 has the following advantages[13]: (1) The insertion of a 315 bp fragment between the BamH I and SalI sites makes it easier to recover the double-digested product and greatly reduces the generation of self-ligated clones. (2) The control protein is no longer required for the SDS-PAGE screening of recombinant (r-) clones expressing the 8/18mer peptides[13]. In our previous study, this method was also used to identify immunodominant epitope BCEs of the Crimean-Congo hemorrhagic fever virus NP[14] and GTV Gn [15]. All BCEs identified were recognized by positive sheep sera of the corresponding virus, thus confirming the reliability of the BSP method and the ease of epitope mapping.

In this study, we used rabbit anti-GTV NP polyclonal antibodies (pAbs) to screen for all minimal BCEs on the NP, performed antigenic identification of the BCEs using natural positive sheep sera with anti-GTV antibodies, and compared the identified BCEs to the closely related SFTSV strain by amino acid sequence alignment to determine the conservation of the BCEs. Then, secondary structure prediction and 3D structure analysis were performed for the BCE sequences using DNAstar11.0 and PyMOL. Our findings

will serve as basic data for further elucidating the biological characteristics of GTV NP and the development of GTV multi-epitope virus detection reagents and vaccines.

Results

Antigenic epitope mapping of GTV NP segment. Based on the improved BSP strategy, we first synthesized 30 16mer oligonucleotides with 8 overlapping amino acid residues spanning the full length of GTV-NP, which were individually fused with the GST188 tag and inserted into pXXGST-3[13]. The SDS-PAGE results indicated that all biosynthesized 16mer-peptides were expressed correctly. The antigenicity of the NP segment was determined by western blotting using rabbit anti-GTV-NP pAbs. The results showed that four 16mer-peptides (i.e., NP11, NP21, NP24, and NP30) reacted specifically with rabbit anti-GTV-NP pAbs, thus suggesting the presence of antigenic sites or epitopes on the NP (Fig. 1).

Mapping of GTV-NP fine epitope motifs. To further refine and map the epitopes on the NP segment, the positive peptides described above were divided into 8mer-peptides with 7 overlapping amino acid residues[13](Fig. 2). SDS-PAGE indicated that all synthetic peptides could be expressed correctly (Fig. 3a, 3c, 3e, 3g), and western blot showed that a total of five 8mer-peptides could bind specifically to rabbit anti-GTV-NP pAbs. Figure 3b shows that the pAbs could recognize the 8mer-peptides NP11-3 (^{83}IMALQEKY^{90}) and NP11-8 (^{88}EKYGLVER^{95}) derived from the 16mer-peptide NP11, which indicates that the minimal motifs for the NP11 epitope are IMALQEKY (named E-np-1) and EKYGLVER (named E-np-2). Similarly, the minimal epitope of NP21 is IMALQEKY (named E-np-3) (Fig. 3d); and those of NP24 are IMALQEKY (named E-np-4) and IMALQEKY (named E-np-5) (Fig. 3f).

Mapping of the NP30 minimal epitope motif with 9mer-peptides. Since the 8mer-peptides derived from NP30 could not react specifically with rabbit anti-GTV-NP pAbs, we proceeded to construct 4 sets of 9mer-peptides with 8 overlapping amino acid residues using the BSP method. SDS-PAGE showed that all 9mer-peptides were expressed correctly (Fig. 3H), and western blot showed that rabbit anti-GTV-NP pAbs could recognize the 9mer-peptide NP30-4 (236ETAAAYRNL245) derived from the 16mer-peptide NP30 (Fig. 3H). This suggests that the minimal motif of the NP30 epitope was ETAAYRNL (named E-np-6). Thus, a total of six minimal BCEs were identified in the NP segment.

Reactivity of GTV-positive sheep sera with BCEs. Western blot analysis was performed on the six selected BCEs using sheep sera infected/uninfected with GTV to determine whether other host antisera could recognize the BCEs that had been recognized explicitly by rabbit anti-GTV-NP pAbs. All six BCEs were able to react with GTV-positive sheep sera (Fig. 4a), while none reacted with GTV-negative sheep sera (Fig. 4b). These results suggest that both rabbits and sheep could recognize these six BCEs, and the six minimal BCEs (i.e., E-np-1, E-np-2, E-np-3, E-np-4, E-np-5, and E-np-6) could be used as candidate BCEs.

3D conformation and sequence conservation analysis of minimal epitope motifs. NP amino acid sequence alignment was performed between the GTV DXM strain (ALQ33263.1) used in this study and eight SFTSV strains from different countries and regions to analyze the conservation of the selected

BCEs (Fig. 5). The selected SFTSV strains represented nine genetic lineages: C1 (China, BAQ59293.1), J1 (Japan, BBD19940.1), C2 (China, AFJ44285.1), C3 (China, AGI97126.1), C4 (China, AGM33040.1), C5 (China, AGM33263.1), K1 (Korea, AKI34303.1), and K2 (Korea, ASW22989.1). Sequence analysis showed that the six BCEs had a high degree of identity with the corresponding sequences of the eight strains. Also, epitope E-np-2 was fully conserved and could be used as a candidate for broad-spectrum multi-epitope vaccine design, while the alignments of E-np-1, E-np-3, E-np-4, E-np-5, and E-np-6 exhibited difference in only one amino acid in GTV DXm sequence compared with the that of corresponding sequences. For example, epitope E-np-1 (^{83}IMALQEKY^{90}) had mutated from "L" to "I^{83}" in the GTV DXM strain; epitope E-np-3 (^{162}TTKILMEA^{169}) had mutated from "T" to "I^{165}" in the GTV DXM strain; the epitopes E-np-4 (^{187}GASKAEVY^{194}) and E-np-5 (^{191}AEVYNSFR^{198}) had mutated from "T^{191}" to "A^{191}" in the GTV DXM strain; epitope E-np-6 (^{236}ETAAAAYRNL^{245}) had mutated from "T^{191}" to "A^{191}" in the GTV DXM strain, and epitope E-np-6 (^{236}ETAAAAYRNL^{245}) had mutated from "V" to "T" in the GTV DXM strain and from "V" to "A" in the Japanese strain (BBD19940.1). **Fig. 5. Sequence comparison between GST-NP and 8 SFTSV strains.** The GenBank codes and sources are shown at left and the sequence analysis was based on the ClustalW program. The six of fine BCEs and APs recognized by pAbs are highlighted, and the variable aa residue within the BCE motif are highlighted in red Dots

To further determine whether BCEs with amino acid differences in Fig. 6 could be used as universal diagnostic reagents, we synthesized five mutant peptides based on the amino acid mutations in the different epitopes: vE-np-1 (LMALQEKY), vE-np-3 (TTKTLMEA), vE-np-4 (GASKTEVY), vE-np-5 (TEVYNSFR), and vE-np-6 (EVAAAAYRNL), and their antigenicity was analyzed using western blot. These five mutant peptides were tested for antigenicity based on the mutation from I^{83} to L in E-np-1, from I^{165} to T in E-np-3, from A^{191} to T in both E-np-4 and E-np-5, and A^{237} to in E-np-6. The results showed that all mutant 8mer-peptides could react with positive sheep serum but not with negative sheep serum (Fig. 6), thus indicating that all six BCEs could be candidates for universal diagnostic reagents and broad-spectrum multi-epitope vaccine design.

The secondary structure of GTV-NP was predicted using DNAstar to show its antigenicity and hydrophilicity, while its 3D structure was simulated using PyMOL™ to locate all selected BCEs. Each BCE was labeled with a different color, among which E-np-1 overlapped E-np-2 by three amino acids, and E-np-4 overlapped E-np-5 by three amino acids, and the overlapping amino acids are displayed in yellow (Fig. 3). The results showed that all BCEs were located on the NP structure surface (Fig. 7A and 7B), which suggests that they had good surface accessibility and hydrophilicity. This is consistent with the secondary structure prediction (Fig. 7C), which shows that they are accessible for antibody chimerization and favorable for antibody-specific binding.

Discussion

As an emerging bunyavirus, the genus Banyangvirus contains several necessary pathogens that pose a severe public health risk[3]. This includes SFTSV, which can cause severe hemorrhagic fever-like illness

with a mortality rate of up to 30%[16], and has been added to the World Health Organization (WHO) list of priority pathogens in need of attention[17]. Since HRTV was first reported in 2009, 10 HRTV infected patients have been recorded, including three cases of death[18]. Several other neglected strains of Banyangvirus have been identified elsewhere globally, which have been associated with fever or non-specific illness[19–21]. GTV is the third representative strain of this genus, and the presence of antibodies against this virus has been confirmed among residents in the Wusu region of Xinjiang, suggesting that this may be harmful to the local population[5]. There are currently no licensed vaccines or specific antiviral drugs against Banyangvirus. Therefore, identifying the antigenic epitopes of viral structural proteins and analyzing their conservation can increase our understanding of the antigenic structure and immunogenicity of Banyangvirus, which is essential for the future development of Banyangvirus assay and vaccine.

The structural protein NP is the most abundant in many viruses and has a crucial impact on viral replication. Recombinant NPs have been used for serological diagnostics and monoclonal antibody preparation for several viruses, such as EBOV and SFTSV[22,23]. For bunyaviruses, NPs are the most abundant viral products in virions and virus-infected cells[24]. The NP of RVFV is reported to be an immunodominant viral protein, and anti-NP antibodies are readily detected in the early post-infection and recovery phases[25]. The present study examined the antigenic epitopes of GTV NP, which will help monitor the early diagnosis and prevention of diseases that may be caused by GTV.

Long epitope peptides of a protein antigen may cross-react with antibodies of other antigenic proteins, and thus, identifying the minimal motifs of protein antigen epitopes can improve the specificity of virus detection. The improved overlapping BSP strategy adopted in this study is simple, efficient, reliable, and versatile and can directly identify the minimum BCE motifs of the target proteins[13]. Using this method, we screened a total of six minimal BCE motifs on GTV NP, namely E-np-1, ^{83}IMALQEKY^{90}; E-np-2, ^{88}EKYGLVER^{95}; E-np-3, ^{162}TTKILMEA^{169}; E-np-4, ^{187}GASKAEVY^{194}; E-np-5, ^{191}AEVYNSFR^{198}; and E-np-6,^{236}ETAAAAYRNL^{245}, all of which were shown to react with natural GTV-positive sheep serum in western blot assay. These epitope findings can serve as basic data for the future development of GTV-specific detection methods.

Understanding the similarity and specificity of immune responses to target antigens in different species is one of the essential goals of epitope identification studies. Therefore, the present study compared the antigenicity of rabbit and sheep anti-GTV sera to NP. The results showed that the six BCEs identified by rabbit anti-GTV NP pAbs could react with sheep sera that were naturally infected with GTV, thus indicating that the immune recognition of GTV-NP was similar in rabbits and sheep. Previous studies have shown that although rabbits and sheep are biologically and genetically very different, their immune systems can still recognize most or all the epitopes of a given antigen[14,26,27]. This also explains why rabbit serum can identify BCEs for various viral proteins[28]. However, differences in the immune recognition of specific BCEs between humans and rabbits or sheep have rarely been reported. Thus,

whether the BCEs identified in this study can also be recognized by human sera (sera from GTV patients are currently lacking) is a question worth exploring and requires further research.

Multiple sequence alignment of the six BCEs identified on GTV NP with eight SFTSV strains was performed using the DNAman software, which showed that the six BCEs had 80%-100% sequence identity with the corresponding SFTSV sequences. The mutant peptides vE-np-1 (LMALQEKY), vE-np-3 (TTKTLMEA), vE-np-4 (GASKTEVY), vE-np-5 (TEVYNSFR), and vE-np-6 (EVAAAAYRNL) were synthesized based on the results of different amino acid mutations in different epitopes, and western blot analysis was performed using positive sheep sera. The results showed that all mutant peptides reacted with positive sheep serum, whereas negative sheep serum did not recognize the mutant 8mer-peptides (Fig. 6). This indicates that the minimal antigenic epitope motifs were recognized by the antiserum despite the differences in individual amino acids, which implies that the mutations did not significantly affect the recognition of these epitopes. Since there is no reliable crystal structure for GTV NP, we can only speculate using the crystal structure of SFTSV NP, which has the highest similarity. Mapping our six BCEs to the crystal structure of SFTSV NP revealed that these epitopes are located on the surface of the protein structure and are not part of the critical sites and surrounding regions that bind with viral RNA (e.g., G^{65}, N^{66}, K^{67}, I^{181}, P^{127}, and F^{177}), hence slight mutations may occur[29]. This also explains why GTV NP can replace SFTSV NP in viral replication[5]. Since these sites are located on the protein surface, and small mutations are not sufficient to affect the recognition of SFTSV and GTV by BCEs, they can be used to produce broad-spectrum antibodies against GTV and SFTSV. It has been shown that monoclonal antibodies/antibodies against bunyavirus NPs partially protect the host against virulent viruses[30–32]. Therefore, more in-depth studies are needed in the future to determine whether antibodies against GTV NP have protective effects.

With the recent development of bioinformatics, epitope analysis of antigenic proteins has been applied extensively in epitope prediction, substantially reducing the laboratory workload and avoiding the blindness of traditional epitope studies[33]. The method has also been shown to have excellent reliability in previous studies conducted by our group. We analyzed the hydrophilic and antigenic regions of NP linear BCEs and showed that all the identified BCEs were located in the hydrophilic region (Fig. 7C). When these identified BCEs were identified in the constructed 3D model, it was observed that they occupied a relatively large and accessible surface area (Fig. 7A and B) in the antibody-binding region, which is as hypothesized. This suggests that antigenic epitope prediction tools are reliable and can reduce the workload and cost of epitope mapping in immunodiagnostics and accurate BCE motif identification[34].

In summary, this study identified a total of six BCEs using rabbit anti-NP pAbs, all of which were recognized by sheep antiserum infected with GTV. They were also highly conserved with the homologous protein sequences of representative SFTSV strains. These results will improve our understanding of the antigenic properties of GTV-NP and contribute to the further development of GTV multi-epitope detection reagents and prophylactic vaccines.

Material And Methods

Ethics statement. The study was approved by the Committee on the Ethics of Animal Experiments of Xinjiang Key Laboratory of Biological Resources and Genetic Engineering (BRGE-AE001), Xinjiang University. All methods were performed in accordance with the relevant guidelines and regulations. The animal serum samples were collected using random sampling and this process did not involving killing the animals. All sections of this report adhere to the ARRIVE Guidelines for reporting animal research[35].

Plasmids and antibodies. The recombination (r-) plasmid of pET-32a-GTV-NP (aa 1-285) was previously constructed and stored by our research group. The prokaryotic expression plasmids of pXXGST-3 expressing short peptide fusion protein were kindly provided by Professor Wan-xiang Xu from Shanghai Institute of Planned Parenthood Research. Rabbit Polyclonal antibody (pAbs) against GTV-Gn was kindly donated by Professor Fei Deng from Wuhan Institute of Virology, Chinese Academy of Sciences[5]. Escherichia coli BL21 (DE3) cells were used to express 16/8mer peptides fused with a truncated GST188 protein (i.e., with the initial 188 aa of GST)[28].

Other reagents and materials. DNA ligase and restriction enzymes BamH I and Sal I (Takara Co., Ltd, Dalian, China), QIA quick Gel Extraction Kit (QIAGEN, Duesseldorf, Germany), unstained or pre-stained molecular weight markers (Thermo Fisher Science, Waltham, MA, USA), 0.2 μm nitrocellulose membrane (Whatman GmbH, Dossel, Germany), and Western blotting detection kit (GE Healthcare,Buckinghamshire, UK).

Biosynthetic short peptides. To screen epitope mapping of NP from GTV strain DXM (GenBank accession number: ALQ33263.1) using a modified overlapping biosynthetic peptide (BSP) method[12], we used the feasible strategy shown in Fig. 8, the NP with 245 amino acid (aa) residues was first truncated into thirty of overlapping 16 mer-peptides. The 16mers all had an overlap of 8 aa residues between each two adjacent peptides. Then the reactive 16mer-peptides shown in Western blotting will be further shortened into several sets of 8mer peptides with an overlap of 7 aa each other, as well as the overlapping longer 9 mer-peptides were designed for the reactive 16 mer-peptides failed to find any reactive band of 8 mer-peptides. The aa sequences of expressed 16/8mer peptides and their positions on Gn were shown in S1 and S2 Tables.

The DNA fragments encoding these short peptides were synthesized by Wuhan Tianyi Huiyuan Biotechnology Co., Ltd. Each fragment contained a *Bam*H I site at the 5'end, a *Sal* I cleavage site linked with a TAA termination codon at the 3' end. All DNA fragments encoding each short peptide were cloned into the pXXGST-3 vector expressing GST188-tagged protein in E. coli, respectively[12,28].

Expression of designed short peptides. The constructed plasmid expressing overlapping peptides were transformed into E.coli BL21 (DE3) competent cells. The expression of each short peptide fusion protein was thermally induced at 42˚C after mass-rearing of BL21[15]. To screen r-clones, the collected cell total proteins from each induced clone were analyzed by SDS-PAGE, and then those confirmed r-clones were

sent to Ikang Biosciences Co., Ltd. for DNA sequencing. Finally, each collected cell pellets containing target peptide fusion protein was stored at -20˚C, respectively.

SDS-PAGE and Western blot. The collected cell total proteins were separated by SDS-PAGE gel electrophoresis after thermal induction. The gel was stained with Coomassie brilliant blue R-250 for analyzing the band corresponding to the target 16/8mer peptide, and the peptides were used for Western blotting by electrotransferring onto a 0.2 μm nitrocellulose (NC)[36,37], incubated with rabbit anti-NP Abs (1:2500 dilution) or sheep sera (1:100 dilution) as the primary antibody (Positive or negative sheep anti-GTV sera were identified by immunofluorescence method at Wuhan Institute of Virology, Chinese Academy of Sciences), and then reacted with goat anti-rabbit IgG or mouse anti-goat IgG conjugated to HRP (1:5000 dilution) as the secondary antibody. Finally, the blot was performed using the LAS-4000 hypersensitive chemiluminescence imager (Japan)[13].

Sequence analysis and 3D modeling. To analyze the similarity of each mapped BCEs among homologous proteins, the NP sequences of SFTSV strains from different countries and genetic lineages were downloaded from GenBank based on the phylogenetic tree of SFTSV strains[38]. The location of experimentally identified BCEs in the three-dimensional structure (3D) of the Gn protein was analyzed by PyMOL™ software (https://pymol.org/2/). The prediction of secondary structure was based on Garnier and Robson[39] as well as Chou and Fasman[40]. The hydrophilic scheme, flexible regimen, surface accessibility regimen and antigenicity index were analyzed and predicted using the methods of Kyte-Doolittle[41], Karplus-Schulz[42], Emini[43] and Jameson-Wolf[44].

Data availability statement.

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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Author contributions

S.W., A.L. and S.S.: study concept and design; S.W. and S.S.: drafting of the manuscript; S.W., A.L., A.M., X.Y., Y.L., and S.S.: acquisition of data, analysis and interpretation of data; W.X.: material support; S.S., J.D., Y.L., F.D., Y.Z., and S.S.: critical revision of the manuscript.

Competing Interests

The authors declare that there are no conflict of interests we do not have any possible conflicts of interest.

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Figures

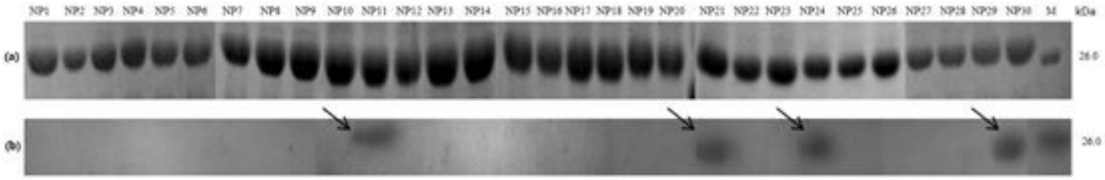

Figure 1

SDS-PAGE and Western blot analysis of expressed 16mer-peptides. (a) SDS-PAGE analysis of expressed 16mer-peptides. The numbers of NP1-NP30 indicate each 16mer-peptide in cell total proteins. The cell proteins of each r-clone were resolved by 12% SDS-PAGE gel electrophoresis and stained with Coomassie brilliant blue. M, the protein molecular marker. (b) Western blot analysis for mapping reactive 16-mer in NP1-NP30. The rabbit antiserum (1:2000 dilution) against GTV-NP was used in Western blotting. The reactive bands in Western blotting were visualized by enhanced chemiluminescence supplement Arrow indicates positive peptide

Peptide items	Amino acids	Position in NP	Peptide items	Amino acids	Position in NP
NP 11-1	KRIMALQE	81-88	NP 21-1	STTKILME	161-168
NP 11-2	RIMALQEK	82-89	NP 21-2	TTKILMEA ← Enp 3	162-169
NP 11-3	IMALQEKV ← Enp 1	83-90	NP 21-3	TKILMEAY	163-170
NP 11-4	MALQEKYG	84-91	NP 21-4	KILMEAYS	164-171
NP 11-5	ALQEKYGL	85-92	NP 21-5	ILMEAYSL	165-172
NP 11-6	LQEKYGLV	86-93	NP 21-6	LMEAYSLW	166-173
NP 11-7	QEKYGLVE	87-94	NP 21-7	MEAYSLWQ	167-174
NP 11-8	Enp 2 → EKYGLVER	88-95	NP 21-8	EAYSLWQD	168-175
NP 11-9	KYGLVERA	89-96	NP 21-9	AYSLWQDA	169-176
NP 24-1	MRGASKAE	185-192	NP 30-1	KAVETAAAY	233-142
NP 24-2	RGASKAEV	186-193	NP 30-2	AVETAAAYR	234-243
NP 24-3	GASKAEVY ← Enp 4	187-194	NP 30-3	VETAAAYRN	235-244
NP 24-4	ASKAEVYN	188-195	NP 30-4	ETAAAYRNL ← Enp 6	236-245
NP 24-5	SKAEVYNS	189-196			
NP 24-6	KAEVYNSF	190-197			
NP 24-7	Enp 5 → AEVYNSFR	191-198			
NP 24-8	EVYNSFRD	192-199			
NP 24-9	VYNSFRDP	193-200			

Figure 2

Determination of mapped epitope motif. Each mapped BCE minimal motif was determined according to the common sequence present in several overlapping peptides recognized by rabbit pAbs against NP, which all reactive peptides were shown in yellow.

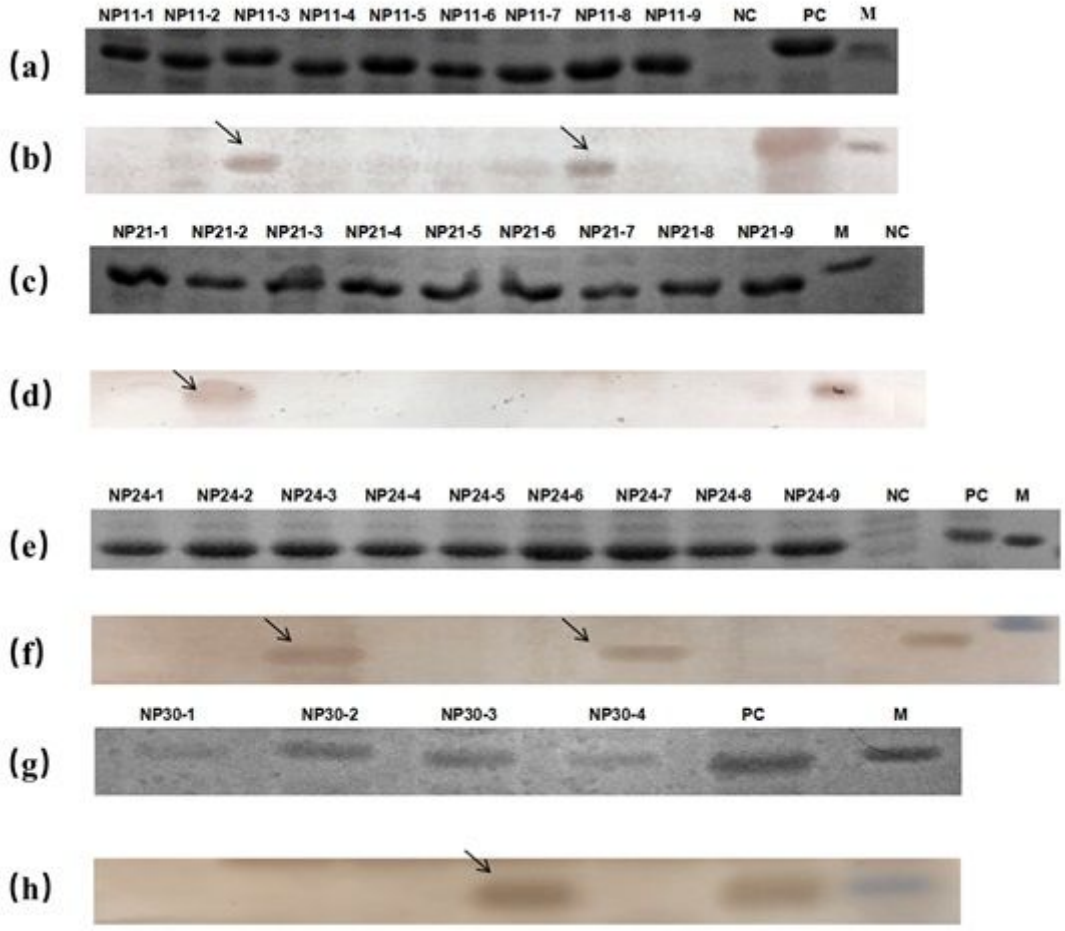

Figure 3

SDS-PAGE and Western blot analysis of expressed 8mer-peptides. (a,c,e,g) SDS-PAGE analysis of expressed 8 mer-peptides. It indicates each short peptide for numbers 8mer NP11-1 to NP11-9 of NP11, NP21-1 to NP21-9 of NP21, NP24-1 to NP24-9 of NP24 and 9mer NP30-1 to NP30-4 of NP30. The cell proteins of each r-clone were resolved by 12% SDS-PAGE gel electrophoresis and stained with Coomassie brilliant blue. M, the protein molecular marker; NC, Negative control of GST188 carrier protein expressed by pXXGST-3; PC, Positive control. (b,d,f,h) Western blot analysis for mapping fine epitopes in each reactive 16mer-peptide. The rabbit antiserum (1:2000 dilution) against GTV-NP was used in Western blotting. The reactive bands in Western blotting were visualized by enhanced chemiluminescence. supplement Arrow indicates positive peptide

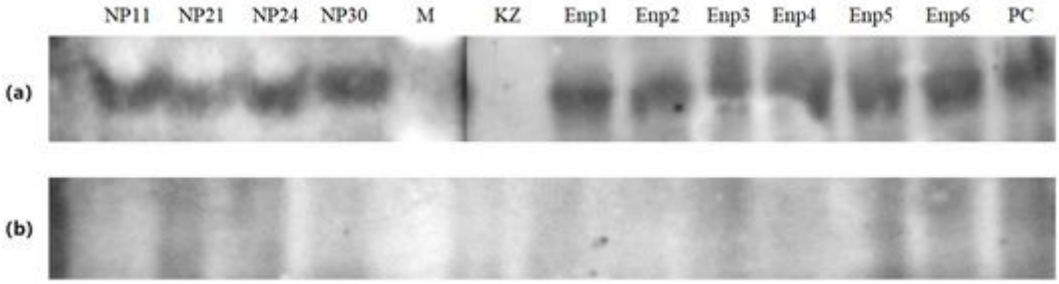

Figure 4

Western blot analysis of mapped BCEs and APs using sheep sera. (a) Using a positive serum from a sheep confirmed GTV-infection. (b) Using a serum from healthy sheep with no history of GTV infection as a negative control. NC, Negative control of GST188 protein. PC, Positive control.

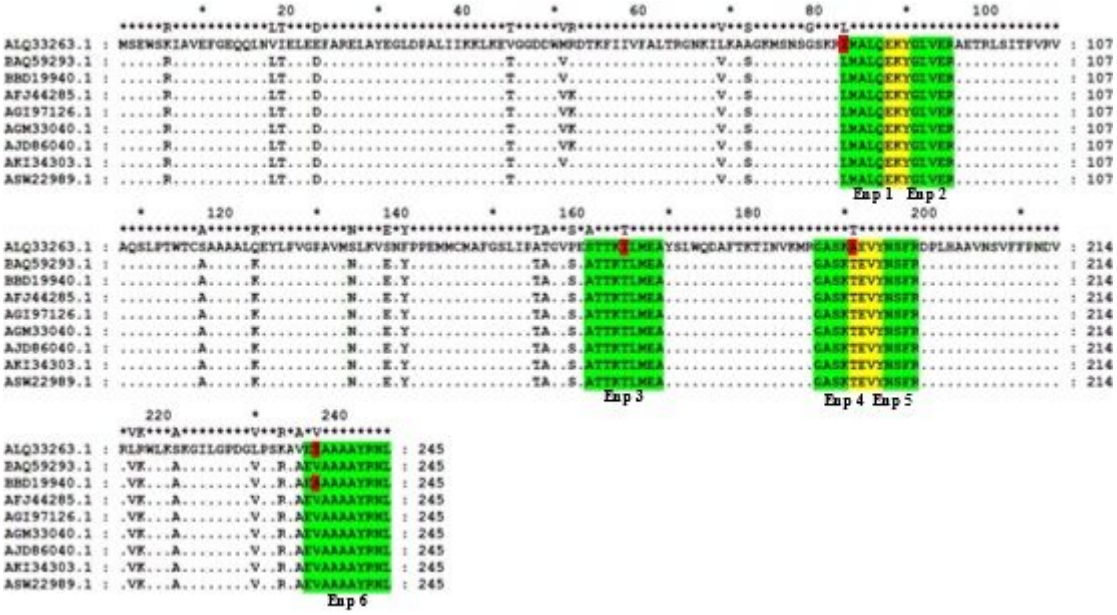

Figure 5

Sequence comparison between GST-NP and 8 SFTSV strains. The GenBank codes and sources are shown at left and the sequence analysis was based on the ClustalW program. The six of fine BCEs and APs recognized by pAbs are highlighted, and the variable aa residue within the BCE motif are highlighted in red Dots

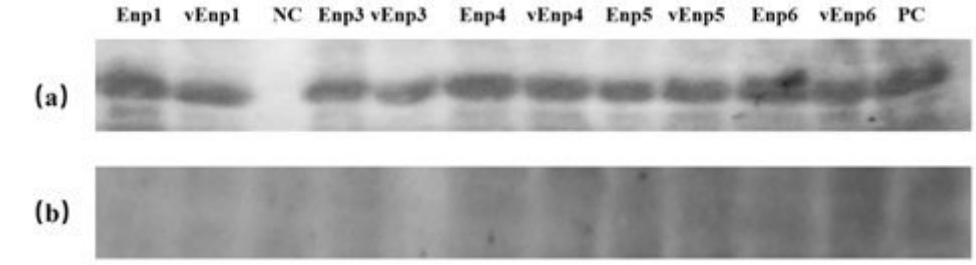

Figure 6

Western blot analysis of mapped BCEs and mutant peptides using sheep sera. (a) Using a positive serum from a sheep confirmed GTV-infection. (b) Using a serum from healthy sheep with no history of GTV infection as a negative control. NC, Negative control of GST188 protein. PC, Positive control.

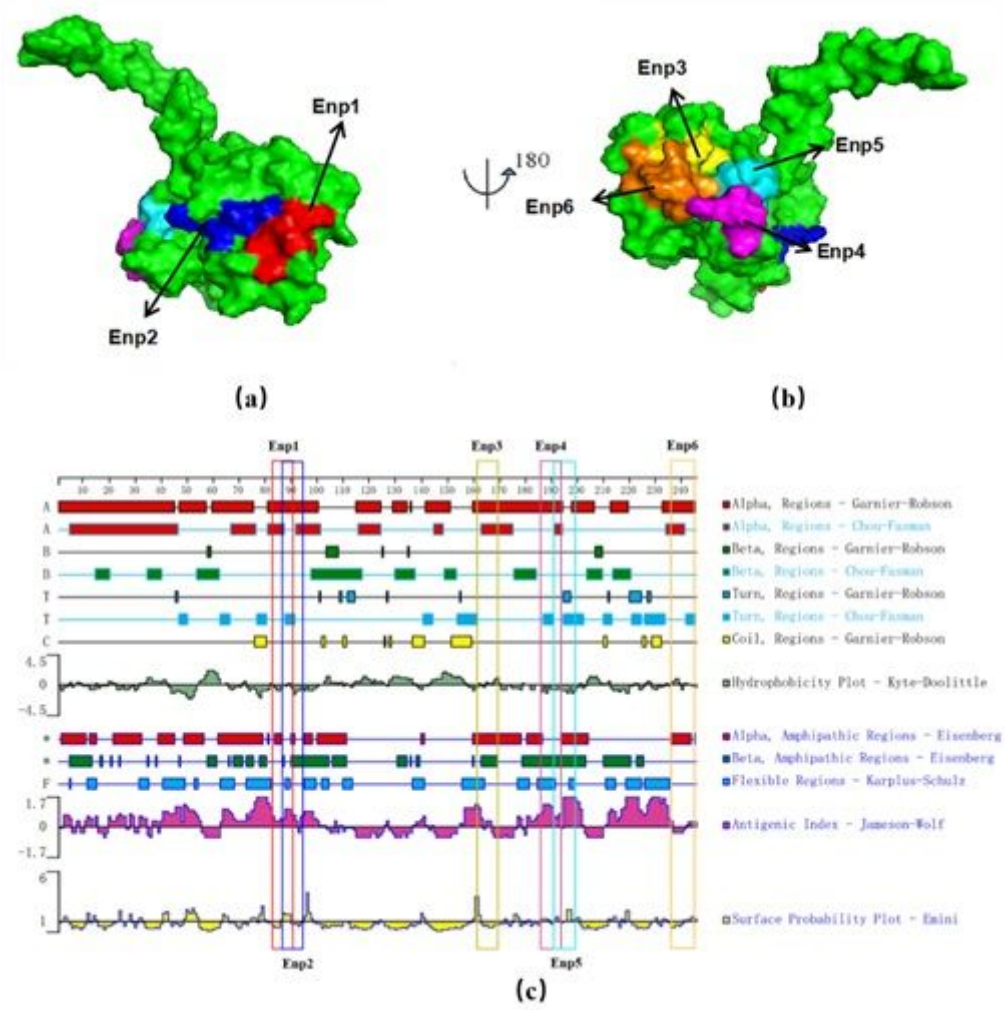

Figure 7

Prediction of Gn secondary structure and 3D localization of each BCEs. (a,b) Location distribution on 3D structure of mapped BCEs and APs on molecular surface were shown in different colors. Enp1 (red), Enp2 (deep blue), Enp3 (yellow), Enp4 (magenta), Enp5 (cyan), Enp6 (orange). The figures were generated using the PyMOL™ molecular graphics system (c) Epitope prediction for GTV-NP using DNAStar-Protean software. The secondary structure, flexibility plot, hydrophilicity, surface probability, and antigenicity index for GTV-NP were taken into consideration.

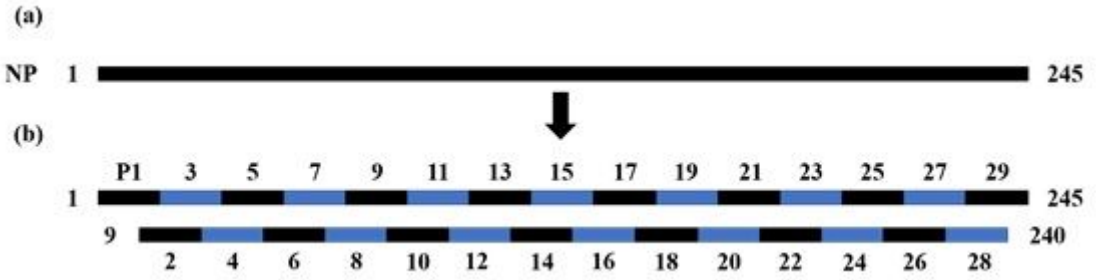

Figure 8

Schematic of epitope mapping strategy. (a) The black band indicates the full- length sequence of GTV-NP. (b) Schematic of epitope mapping strategy involves 29 overlapping 8mer-peptides spanning NP.

Supplementary Files

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