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Genetic Variation Analysis of TGEV Spike Protein Antigenic Sites

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Abstract: The spike genes of 22 different TGEV strains were selected from GenBank and analysed by the Bioinformatics analysis software. The results showed that the A and C antigen sites are more conservative than B and D antigen sites. The results could establish the rationale for preparing vaccine of epitopes and diagnostic reagent.

Key words: TGEV, spike protein, epitope, molecular characteristic, China

INTRODUCTION

Transmissible Gastroenteritis (TGE) is a highly contagious viral disease of swine characterized by vomiting, diarrheaand dehydration. Its causative agent is Transmissible Gastroenteritis Virus (TGEV), considered the principal etiologic agent responsible for dramatic outbreaks of diarrhea and high mortality of newborn pigs in which mortality approaches 100% (Schwegmann-Wessels and Herrler, 2006).

TGEV spike protein is a major viral antigen and plays a crucial role in the induction of neutralizing antibodies. Its binding to the cellular receptor porcine Aminopeptidase N (pAPN) is required or the initial stage of infection. Therefore, the spike protein of TGEV is a good target for vaccine design and antigen detection. It has been documented that four major antigenic sites are located on the aminoterminal half of spike protein, defined C, B, D and A (Ballesteros *et al*., 1997; Izeta *et al*., 1998; Krempl *et al*., 2000; Sanchez *et al*., 1999; Schwegmann-Wessels *et al*., 2003).

Site A is the main inducer of neutralizing antibodies and has been previously subdivided into the three subsites Aa-Ac. Site A contains the residues 538, 591 and 543 which are essential in the formation of subsites Aa-Ac, respectively. In addition, mar mutant 1B.H6 with residue 586 changed had partially altered both subsite Aa and Ab indicating that these subsites overlap in residue 586, i.e., this residue also is part of site A. The peptide 537-MKSGYGQPIA-547 represents at least partially subsite Ac which is highly conserved among coronaviruses. This site is relevant for diagnosis and could be of interest for protection. Other residues contribute to site B (residues 97 and 144), site C (residues 50 and 51) and site D (residue 385). The location of site D is in agreement with PEPSCAN results. Site C can be represented by the peptide 48-P-P/S-N-S-D/E-52 but is not exposed on the surface of native virus. Sites A and B are fullydependent on glycosylation for proper folding while sites C and D are fully or partially independent of glycosylation, respectively.

Once the spike protein has been assembled into the virion, the carbohydrate moiety is not essential for the antigenic sites (Meng *et al*., 2011). In order to illuminate molecular characteristic of TGEV spike protein antigenic sites, we selected 22 strains of TGEV in this study and applied bioinformatics to analyze homology of the amino acid sequence in these antigenic sites. The results could establish the rationale for preparing vaccine of epitopes and diagnostic reagent.

MATERIALS AND METHODS

Amino acid sequence: The spike genes of the 22 different TGEV were selected from GenBank (Table 1). PUR46-MAD, TH98, NEB72-RT and SC-Y had two deletion mutations at 375 aa and 376 aa in contrast with PUR46-MAD and the other strains. In this study, the PUR46-MAD was selected as standard strain.

Analysis of amino acid sequence in antigen sites: The analysis was performed by using DNAMAN and DNAStar. The determiners of A and B antigen sites were determined amino acids. C and D antigen sites were analyzed by determined amino acid and forecast of epitopes imitation.

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Table 1: TGEV strains

Strain names	Separatum	GenBank number	Epitopes composition
TH98	China	AF494337	A1B1C1D3
Attenuated H	China	EU074218	A1B4C1D2
H16	China	FJ755618	A1B4C1D2
TS	China	DQ201447	A1B2C1D2
TSX	China	DQ001167	A1B1C1D2
SC-Y	China	DQ443743	A1B1C1D3
HN2002	China	AY587882	A1B4C1D2
TFI	Taiwan	Z35758	A1B1C1D2
TO14	Japan	AF302263	A1B2C1D1
HKT2	Korea	AF481366	A1B1C1D1
KT2	Korea	AF481360	A1B1C1D1
KT6	Korea	AF481364	A1B1C1D3
133	Korea	AF481365	A1B1C1D1
Purdue	USA	DQ811789	A1B1C1D3
PUR46-MAD	USA	PTGPRCVSF	A1B1C1D3
Purdue P115	USA	DQ811788	A1B1C1D3
Miller	USA	S51223	A1B5C1D2
Miller M6	USA	DQ811785	A1B5C1D2
Miller M60	USA	DQ811786	A1B4C1D1
FS772/70	USA	X53128	A1B2C1D2
96-1933	USA	AF104420	A2B3C2D2
NEB72-RT	Spain	M94099	A1B1C1D3

RESULTS

Analysis of amino acid sequence in antigen site A: The residues 538, 543, 549, 586 and 591 are essential in the formation of site A. In these residues, there are two types, K^{538} G^{543} T^{549} D^{586} R^{591} and K^{538} G^{543} T^{549} N^{586} R^{591} (only in 96-1933). So, the site can be classified into A1 and A2.

Analysis of amino acid sequence in antigen site B: The essential residues in the site are 97, 100, 102, 144, 163 and 165. There are five types, W^{97} R^{100} R^{102} S^{144} S^{163} S^{165}, W^{97} K^{100} R^{102} S^{144} S^{163} S^{165}, D^{97} R^{100} R^{102} S^{144} F^{163} S^{165}, S^{97} K^{100} R^{102} S^{144} S^{163} S^{165} and L^{97} K^{100} R^{102} S^{144} S^{163} S^{165}, named B1-B5, respectively.

Analysis of amino acid sequence in antigen site C: The extent of this linear antigen site is from residue 47 to 55. The essential residue in site C is the residue 51. All the selected strains is S. There are three situations in residue 48, P/S/I and two situations in residue 53, V/A. According to the analytic results by DNA Star software, the changes in P/S (residue 48) and V/A (residue 53) do not alter the antigenicity but when the residue 48 is I, the antigenicity degrades remarkably. This situation only exists in 96-1933 strain. So, there are two types of site C, L-P/S-P-N-S-D-V/A-V-L and L-I-P-N-S-D-V-V-L, named C1 and C2.

Analysis of amino acid sequence in antigen site D: The extent of this linear antigen site is from residue 373 to residue 396 (or residue 398). The essential residues S^{383}, Y384, G^{385} and P^{388} in site D are highly conservative. According to the analytic results by DNAMAN and DNAStar software, the changes in amino acids are complicated and can be classified into eight types but the antigenicity can be classified into three types, named C1, C2 and C3.

DISCUSSION

Before carrying out experiment, using the techniques of bioinformatics to analyze, manipulate, simulate and predict organismic objects could reinforce the possibility of success and applied by more and more researchers. The studies of protein epitopes play a significant role in the theory of immune recognition and the practical application (Wu and Wu, 2000). In this study, the techniques of bioinformatics were used to analyze the homology of amino acid sequence in antigen sites. There were no effectual ways to analyze conformational epitopes such as antigen site A and B of TGEV spike protein.

While the ways of forecasting linear epitopes were comparative successful. Therefore, the determiners of A, B antigen sites were determined amino acids and C, D antigen sites were analyzed by determined amino acid and forecast of epitopes imitation.

The results showed that the antigen sites A, C were highly conservative and antigen sites B, D were diverse. With the development of molecular bioresearch in TGEV more and more researchers paid close attention to the genetically engineering vaccine and diagnostic reagent based on epitopes (Meng and Ren, 2011). Because antigen site A was highly conservative and was a neutralizing epitope, it was a good target for vaccine design. Antigen site C was highly conservative too. Meanwhile, this site was absent in Porcine Respiratory Corona Virus (PRCV) thus, it had special significance in differential diagnosis of TGEV from PRCV.

CONCLUSION

There was a notable strain 96-1933 which had remarkable changes in antigen sites A, C and D in contrast with the other strains. This indicated that neotype strains emerged in some place under the immunoselection. These neotype strains could take negative influence for prevention and cure of TGE and should be caused more attention.

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