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PRODUCTION AND IMMUNOGENICITY OF MULTIPLE ANTIGENIC PEPTIDE (MAP) CONSTRUCTS DERIVED FROM THE S1 GLYCOPROTEIN OF INFECTIOUS BRONCHITIS VIRUS (IBV)

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ABSTRACT

Synthetic peptides were prepared as multiple antigenic peptide (MAP) constructs to the S1 glycoprotein of infectious bronchitis virus (IBV). The MAP system has been used in the production of anti-peptide and anti-protein antibodies. It has an advantage over linking peptides to a highly immunogenic carrier molecule because antibodies are not produced to the MAP core matrix of lysine residues. Two 25-residue peptides were synthesized to the Arkansas serotype and two were synthesized to the Massachusetts serotype of IBV. The peptide sequences correspond to amino acid residues 64 to 88 and to residues 117 to 141 for each of the IBV serotypes. A MAP construct for each peptide was prepared by linking 4 copies of a peptide to the immunogenetically inert core matrix of lysine residues. The MAP constructs were used to immunize specific pathogen free chickens. Anti-peptide ELISA titers and the dot immunobinding assay against the homologous peptide were positive for all of the sera tested whereas the anti-whole virus ELISA titers and virus neutralization titers were negative for all of the sera tested. Hyperimmune sera against whole virus did not cross react with synthetic peptides made to the heterologous virus suggesting a possible role for the MAP constructs in a serotype specific dot blot or ELISA test for IBV.

INTRODUCTION

Peptides have been used experimentally as vaccines to protect against several viral disease agents including foot and mouth disease virus[1], canine parvovirus[2], and mouse

Corona- and Related Viruses, Edited by P. J. Talbot and G. A. Levy
Plenum Press, New York, 1995

hepatitis virus[3,4]. They have also been used to study the structural features of viral immunogenic proteins[5].

A number of algorithms have been developed to predict epitopes in proteins from the amino acid sequence[5,6,7,8]. In general, peptide antigenicity has been correlated with the structural features of the protein. Some of those features include hydrophilicity, amphipathicity, and surface accessibility[8]. In addition, sequence variability has been used to predict epitopes on viral proteins[8].

Several factors need to be considered when designing synthetic peptide vaccines. Since peptides do not generally stimulate an immune response by them selves, they must be conjugated to a carrier molecule to be immunogenic. The two most popular carrier molecules are bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH)[8].

Multiple antigenic peptide (MAP) constructs provide an alternative means of producing immunogenic peptides. First described by Tam[9] in 1988, MAP constructs consist of either four or eight peptides linked by the COOH-terminal amino acid to a core matrix of lysine residues. An advantage of using MAP constructs is that antibodies are not produced to the immunologically inert core matrix of lysine residues. In addition the need to conjugate the peptide to a carrier molecule is eliminated. A disadvantage of using MAP constructs is that it is not possible to use a T-cell stimulating carrier molecule which has been shown to be critical in developing a protective immune response using some peptides[1,10]. However, T-cell stimulating epitopes could be incorporated into the same MAP construct as B-cell epitopes since several peptides can be linked to the same core matrix of lysine residues.

In this study, we investigated the feasibility of developing a vaccine using MAP constructs of synthetic peptides against the S1 glycoprotein of infectious bronchitis virus (IBV).

METHODS

Synthetic Peptides

Two synthetic peptides to the Arkansas 99 (Ark 99) strain of IBV, designated Ark 99A and Ark 99B, and two synthetic peptides to the Mass 41 strain of IBV designated Mass 41A and Mass 41B, were designed based the sequence comparison of the S1 glycoproteins of the two viruses. The sequences of the 25 residue peptides were taken from variable regions between residues 64 to 88 and from residues 117 to 141 (Figure 1). Analysis of the peptides with the Hopp- Woods[7], and the Chou-Fasman[6] algorithms which predicts antigenic determinants based on the hydrophobicity of the sequence and secondary structure respectively showed that each peptide contained hydrophilic regions and a ß pleated sheet and or a α helix.

The 'Fmoc' method was used to synthesize the peptides. Then the peptides were linked to a 4-branch multiple antigenic peptide (MAP) resin (Applied Biosystems, Inc., Foster City, CA) following the manufacturer's instructions.

Immunization Schedule

Five one-day old specific pathogen free chicks per group were immunized with either Ark 99A, Ark 99B, Mass 41A, Mass 41B, Ark 99A and Ark 99B, Mass 41A and Mass 41B, whole Ark99 virus, or whole Mass 41 virus. A negative control group was given sterile saline.The immunization schedule and sera collection times are shown in Table 1. Prior to

A

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                      10          20          30          40          50
Ark 99       1  MLVKSLFLVT  ILFALCSANL  YDNESFVYYY  QSAFRPGHGW  HLHGGAYAVV
Mass 41      1  MLVTPLLLVT  LLCVLCSAAL  YDSSSYVYYY  QSAFRPPNGW  HLHGGAYAVV

                                Peptide A
                      60          70          80          90          100
Ark 99      51  NVSSENNNAG  TAPSCTAGAI  GYSKNFSAAS  VAMTAPLSGM  SWSASSFCTA
Mass 41     51  NISSESNNAG  SSPGCIVGTI  HGGRVVNASS  IAMTAPSSGM  AWSSSQFCTA

                                          Peptide B
                     110         120         130         140         150
Ark 99     101  HCNFTSYIVF  VTHCFKSGSN  SCPLTGLIPS  GYIRIAAMKH  GSRTPGHLFY
Mass 41    101  HCNFSDTTVF  VTHCYK--YD  GCPITGMRQK  NFLRVSAMKN  G-----QLFY
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B

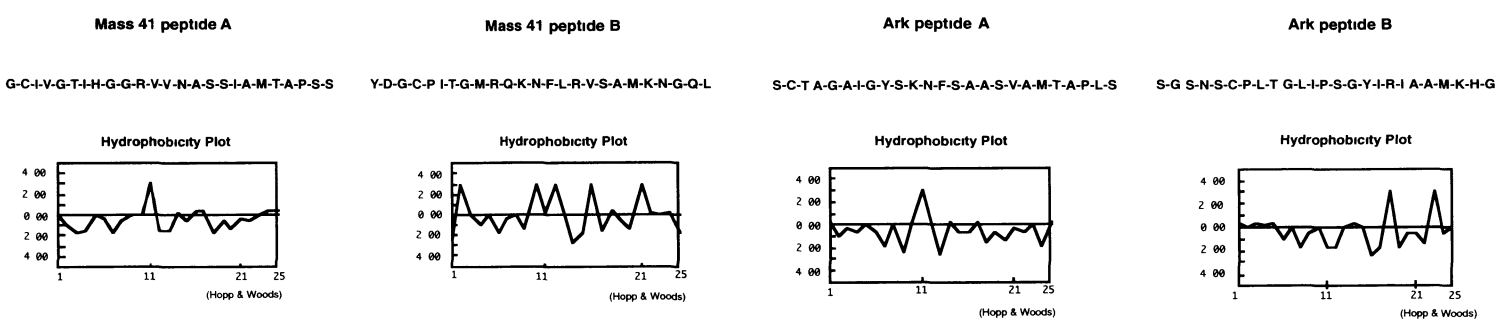

Figure 1. A The relative location of synthetic peptides in the S1 glycoprotein of the Ark 99 and Mass 41 strains of IBV B The peptide sequences and their hydrophobicity plot based on the Hopp and Woods[7] algorithm

Table 1. The immunization[A] schedule and sera collection times for chickens given synthetic peptides to IBV

Age in days	Immunization site	Adjuvant	Sera collection
1	SQ[B]	Complete Freund's	Yes
7	IM[C]	Incomplete Freund's	No
21	IV[D]	None	No
28	IV	None	Yes
35	—	—	Yes

[A]Five specific pathogen free chickens per group were immunized each time with approximately 500 ng of peptide. The birds were immunized with either Mass 41A, Mass 41B, Ark 99A, Ark 99B, Mass 41A and Mass 41B, Ark 99A and Ark 99B, Mass 41 whole virus, or Ark 99 whole virus.
[B]SQ= subcutaneous injection in the back of the neck.
[C]IM= intramuscular injection in the breast muscle.
[D]IV= intravenous injection.

immunization sera was collected from all of the birds and tested for maternal antibodies against IBV by ELISA.

Analysis of Sera

All of the sera collected on days 28 and 35 were tested in a peptide specific ELISA where the appropriate peptide was coated on the plates and by whole virus ELISA where the homologous strain of the virus was coated on the plates.

Sera found to be positive in the peptide specific ELISA test were further tested in immunobinding assays. The dot immunobinding assay was conducted by applying each of the peptides or whole virus onto nitrocellulose filters. Allantoic fluid from non-inoculated embryonating eggs was also dotted onto the filters and served as a negative control.

Western blot analysis was conducted following the procedures of Sambrook *et al.* [11] using sucrose gradient purified whole virus.

The virus neutralization assay was conducted in 10-day old embryonating eggs using standard procedures[12].

RESULTS

Analysis of Sera

The Ark 99A peptide was not soluble and no antibodies were detected by ELISA in birds given that peptide. Thus, it was dropped from the study. Geometric mean titers for the sera from the birds given the other peptides are shown in Table 2. The anti-peptide ELISA titers against the homologous peptide were positive for all of the sera tested and ranged from 400 for the Mass 41A peptide to 6400 for the Mass 41 A&B combination. The anti-whole virus ELISA titers were negative for all of the sera tested.

The virus neutralization titers were negative for all of the sera tested.

Dot immunobinding analysis of the sera positive in the peptide specific ELISA test revealed that the sera reacted with the homologous peptide but did not react with the whole virus (Figure 2). Cross reactivity for some of the sera was observed with the other homolo-

Table 2. Geometric mean titers of sera collected from birds at 35 days post-inoculation with synthetic peptides to the IBV S1 glycoprotein

Group	Anti-peptide ELISA[A]	Anti-virus ELISA[B]	VN[C]
Mass 41A	400	≤ 200	≤2
Mass 41B	1600	≤200	≤2
Mass 41 A&B	6400	≤200	≤2
Ark B	800	≤200	≤2
Ark A&B	6400	≤200	≤2

[A]The homologous peptide was coated on the ELISA plate.
[B]The homologous whole virus was coated on the ELISA plate.
[C]VN= Virus neutralization. Conducted in 10-day old embryonating eggs.

gous peptide but not with the heterologous peptides. Hyperimmune antisera against whole virus reacted with the whole virus and the homologous peptides but not with the heterologous peptides in the dot immunobinding assay.

Western blot analysis (Figure 3) showed that the anti-peptide antibodies in the sera were directed against the S1 glycoprotein of the homologous virus. Only antibodies against the Mass 41B peptide reacted with the heterologous virus.

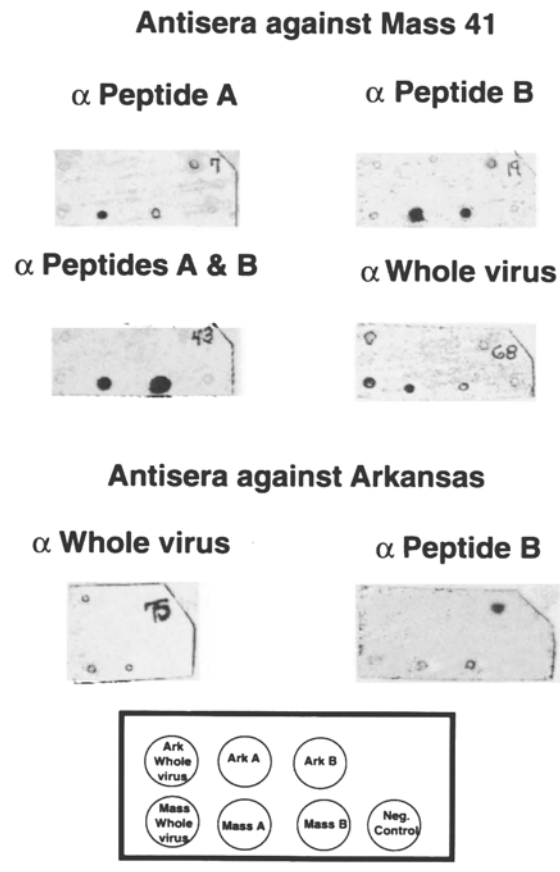

Figure 2. Binding of antibodies to whole virus and MAP constructs prepared to the S1 glycoprotein of IBV. Representative immunoblots for each sera are presented. Antigen placement on the filters is shown in the key at the bottom of the figure. Neg. Control= allantoic fluid from noninoculated embryonating eggs.

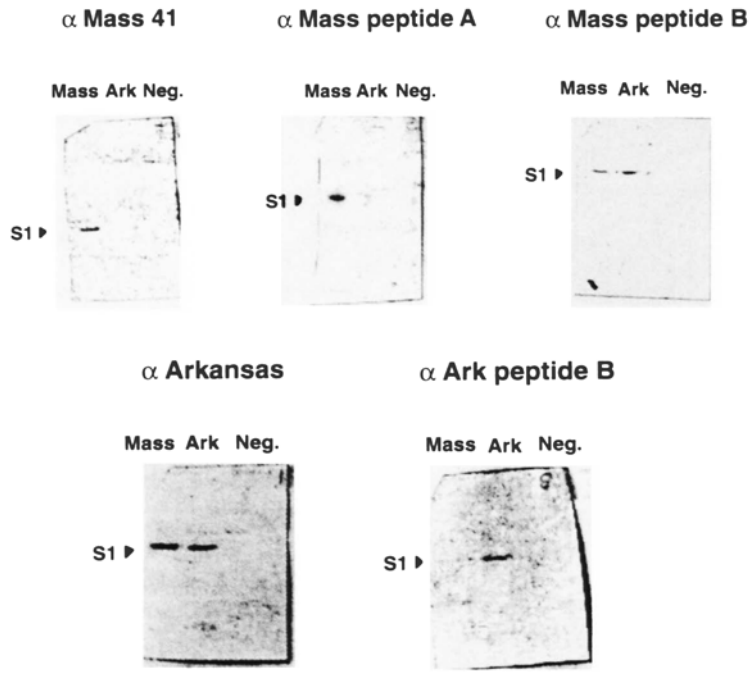

Figure 3. Binding of antibodies to the denatured S1 glycoprotein of IBV Representative Western blots are shown for each sera Neg = allantoic fluid from noninoculated embryonating eggs

DISCUSSION

In this study, synthetic peptides to IBV were prepared as MAP constructs and used to immunize SPF chickens. Sera collected at two different times following immunization contained anti-peptide antibodies and antibodies against the denatured S1 glycoprotein. No antibodies against the native protein or virus neutralizing (VN) antibodies were detected.

Although VN antibodies were not detected in our study, we cannot draw any conclusions regarding the immune status of those birds against challenge with IBV. Studies with foot and mouth disease virus have shown little or no correlation between the presence of *in vitro* neutralizing antibodies and protection following immunization of cattle with synthetic peptides[1]. Those researchers concluded that T-cells were responsible for the protection observed in cattle and that helper T-cells must be stimulated to get protection.

Empirical rules for the prediction of peptide antigenicity and T-cell epitopes from the amino acid sequence of a protein have been developed[8]. Unfortunately the methods do not take into consideration the individual's histocompatibility antigens (MHC) and thus, are not absolutely fool proof[4,8,13]. Researchers are still left with a trial and error approach to developing immunogenic peptides. We designed our peptides based on sequence variability between the Ark 99 and Mass 41 strains of IBV. Computer analysis of the hydrophobicity and secondary structure of the peptides indicated that each peptide contained hydrophilic regions and a ß pleated sheet and or a α helix. It was hoped that the MAP constructs of those peptides would generate VN antibodies in chickens. Perhaps a better immune response could have been generated with the peptides linked to a carrier molecule. Particularly a T-cell stimulating carrier such as ovalbumin or sperm whale myoglobulin which may be recognized by a greater number of histocompatibility types[1,10].

In our study it should be noted that hyperimmune sera against whole virus did not cross react with synthetic peptides made to the heterologous virus in the dot immunobinding assay This result suggests a possible role for synthetic peptides prepared as MAP constructs in a serotype specific dot blot or ELISA test for IBV

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