

(大綱)

Signal Sequence Contributions to the Efficacy of PEDV Subunit Vaccines: Mechanistic Insights and Immunological Benefits

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Abstract

The signal peptide (SP) sequence can potentially enhance a recombinant antigen's antigenicity and protective efficacy. Studies have demonstrated that a lipobox in the signal sequence promotes lipid attachment, which in turn facilitates the activation of Toll-like receptor 2 (TLR2). A small antigenic domain mCOE 500-799 of the Porcine Epidemic Diarrhea Virus (PEDV) spike protein was engineered both with (SP-mCOE) and without (mCOE) the signal sequence. These constructs were assessed in vitro and in vivo. In vitro studies utilized HEK-Blue™ hTLR2 cells to evaluate NF-κB activation upon stimulation with SP-mCOE and mCOE proteins, revealing that SP-mCOE proteins induced a higher level of NF-κB activation compared to mCOE proteins. In vivo, immunized mice exhibited higher antibody levels and a more robust T-cell response in the SP-mCOE vaccine group compared to the mCOE group. Furthermore, the virus neutralization assay revealed that the SP-mCOE group had significantly higher neutralizing antibody titers. These findings confirm that incorporating the native signal sequence enhances the protective efficacy against PEDV and may function as a vaccine adjuvant. The SP-mCOE construct is suitable for further vaccine development and shows promise as a PEDV vaccine candidate.

Keywords: Signal peptide, PEDV, Spike, TLR2



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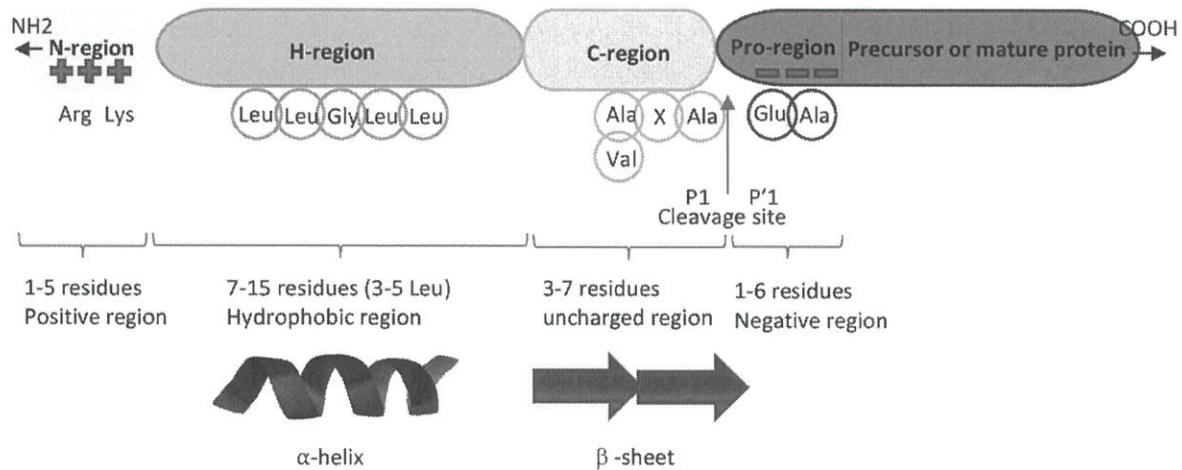
Signal Sequence Contributions to the Efficacy of PEDV Subunit Vaccines: Mechanistic Insights and Immunological Benefits

Student: Haroon Afzal

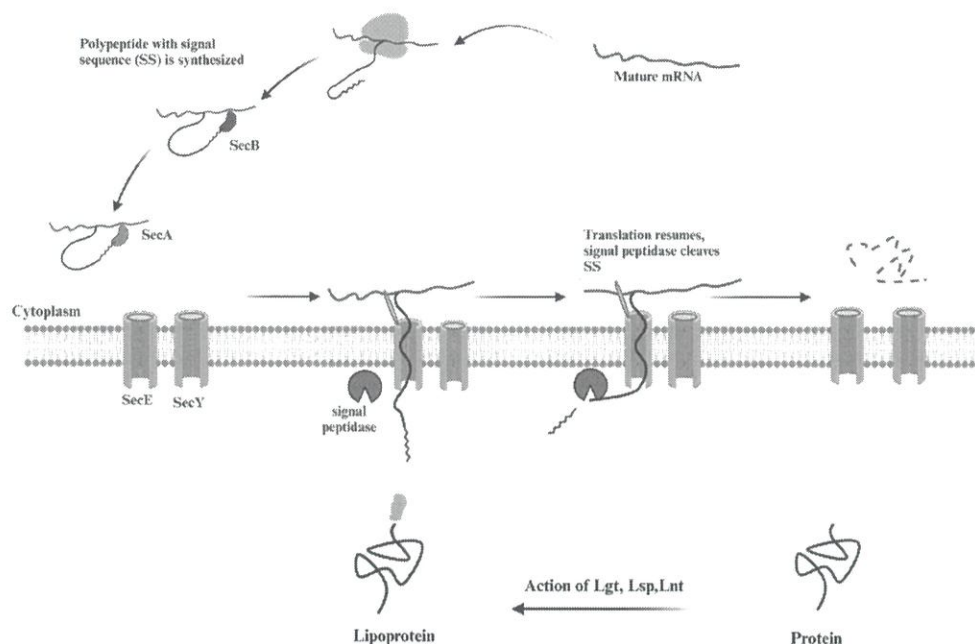
Advisor: Dr. Li-Ting Cheng

Introduction

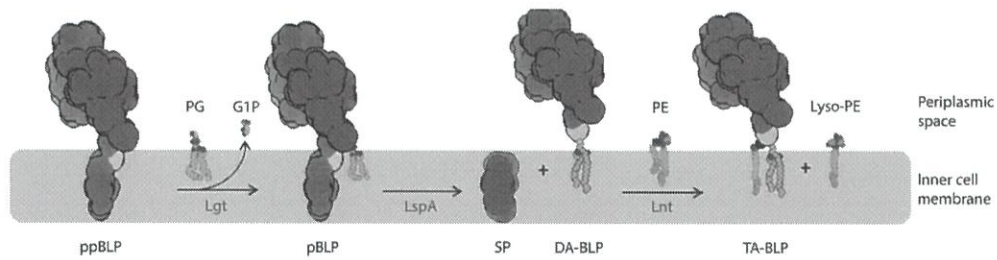
Characteristics sequence and structure in a signal Peptide



Synthesis and export of protein outside cell



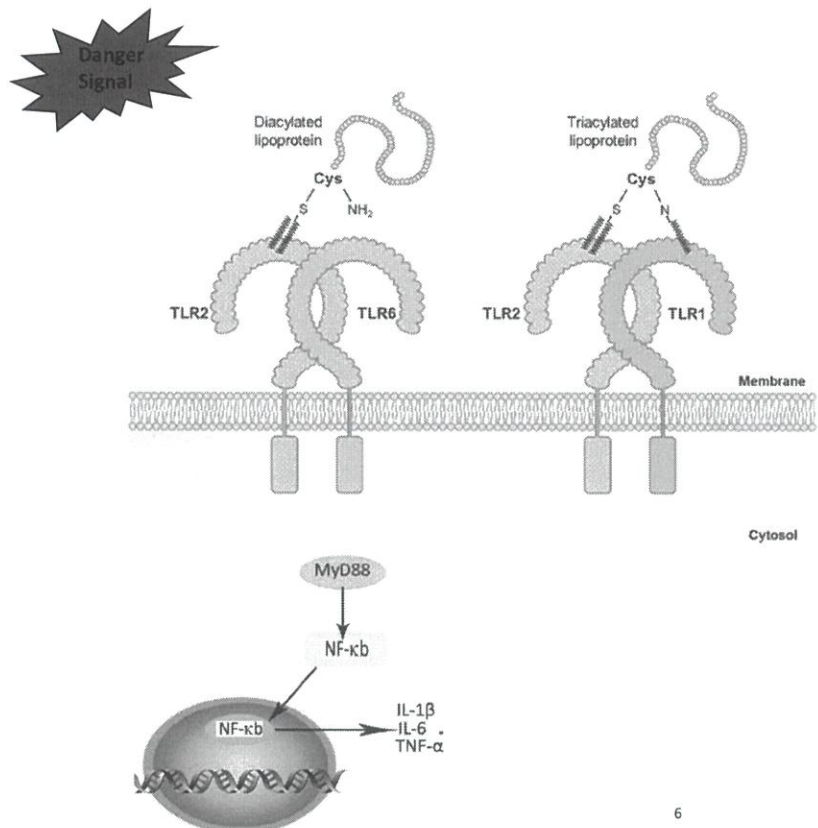
Use of Signal peptide as adjuvant in PEDV subunit vaccine



Process of signal peptide cleavage and bacterial lipoprotein production

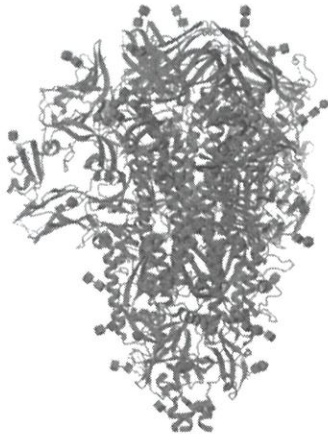
5

Bacterial Lipoproteins and their interaction with TLR2



6

PEDV Spike protein and selection of immunogenic mCOE domain



PEDV Spike Protein (1383aa)



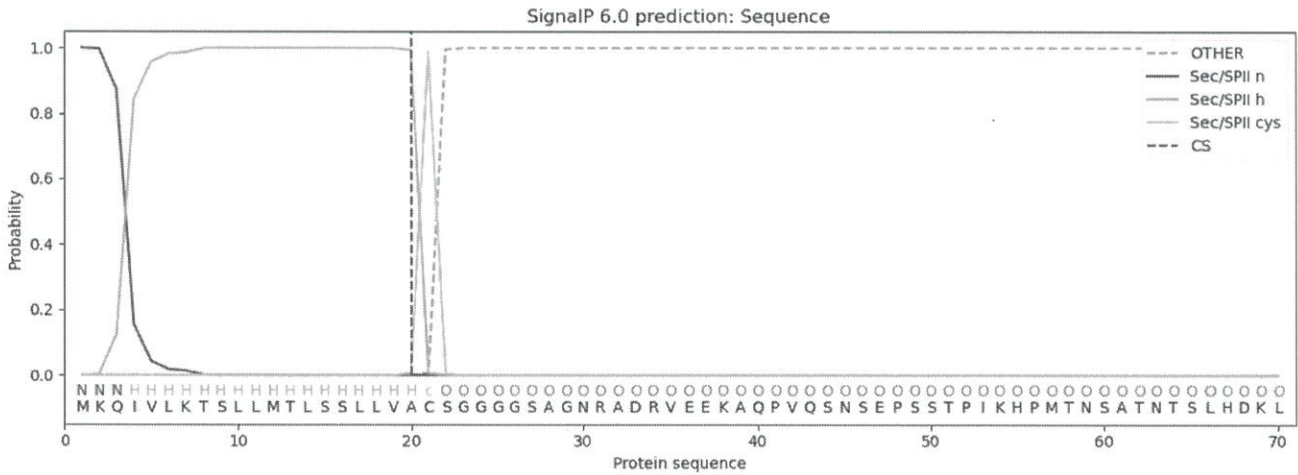
mCOE (499-799aa)



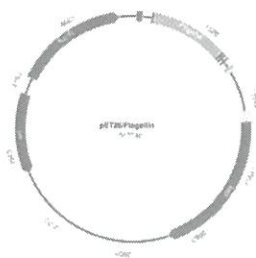
Methods



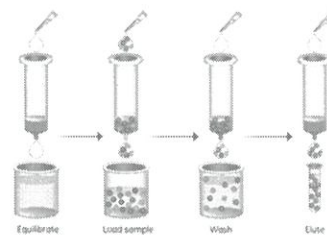
PlpE signal peptide sequence confirmed by SignalP-6.0 Software



Experimental Plan (Adjuvant Preparation)

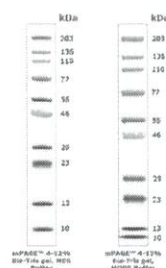


SP-mCOE, COE sequences were synthesized into pET28a



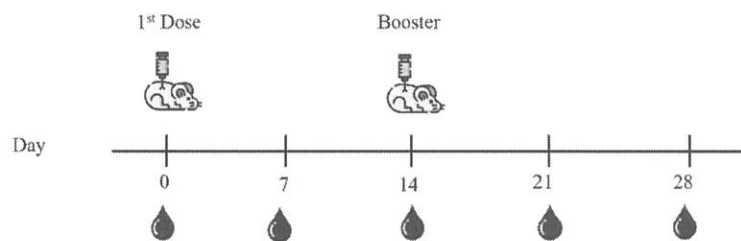
Expressed in *E.coli*, purify by His-Tag affinity chromatography

Confirm by SDS-PAGE and western blotting



Vaccine Formulations and Immunization

	Antigen	Adjuvant	Oil Adjuvant
1	mCOE	Signal Peptide	ISA 206 W/O/W
2	mCOE	Signal Peptide	Nil
3	mCOE	Nil	ISA 206 W/O/W
4	PBS	Nil	Nil



Immune Evaluations



Innate Immune analysis:
Cytokine Assay



Cellular Immunity: CD4⁺/CD8⁺
ratio

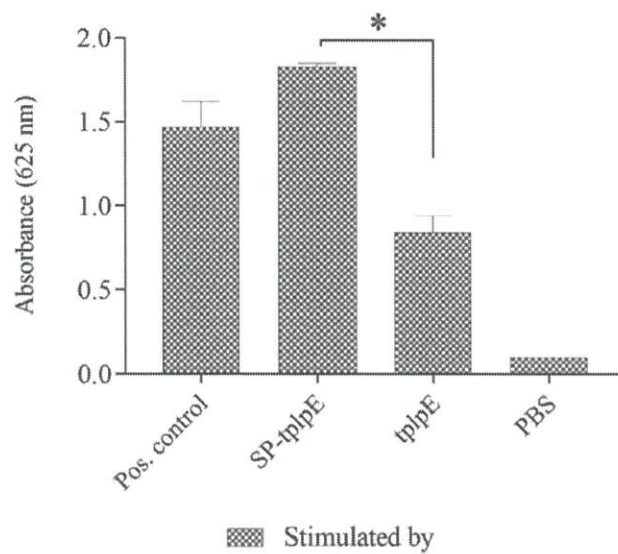


Humoral Immunity: Total IgG
titers, Virus Neutralization Assay

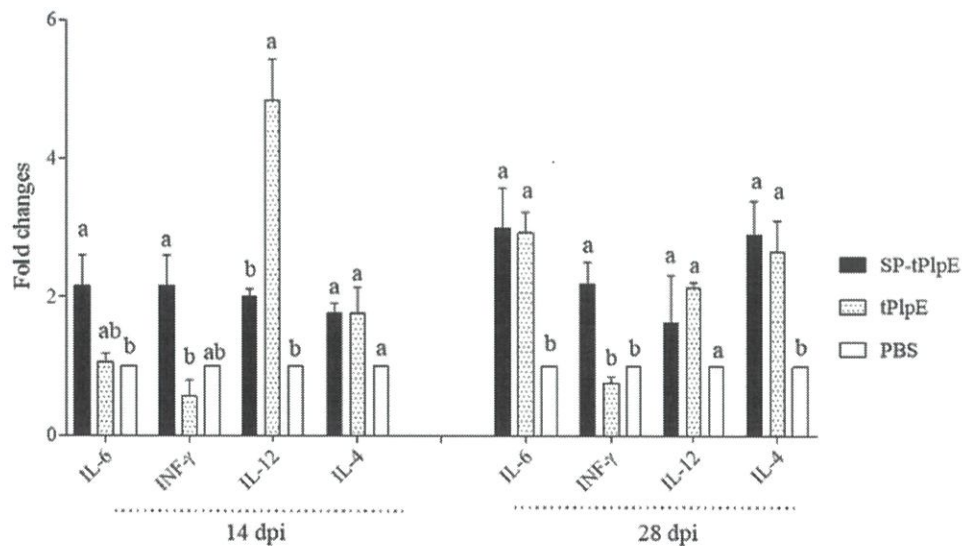
Results

(Include results from our previous study)

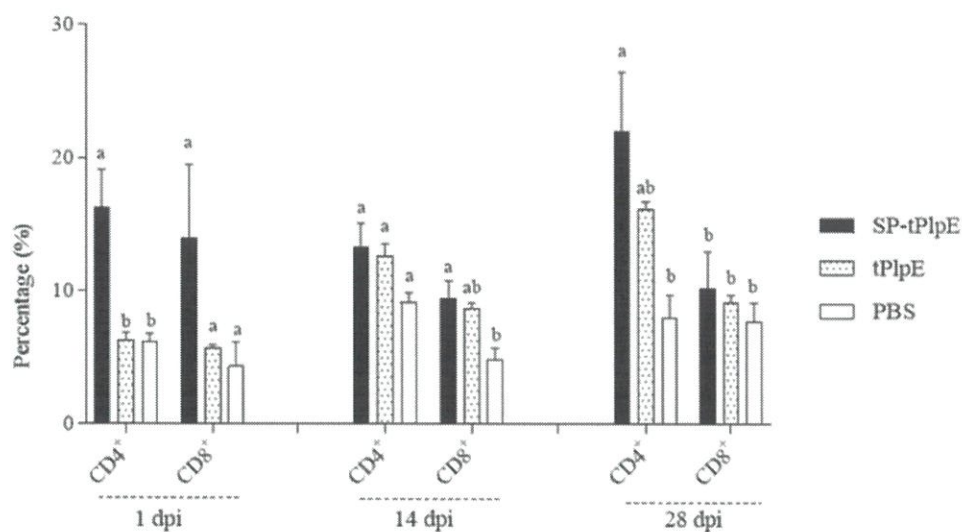
SP-tPlpE Activated TLR2 in HEK-Blue-hTLR2 Cells



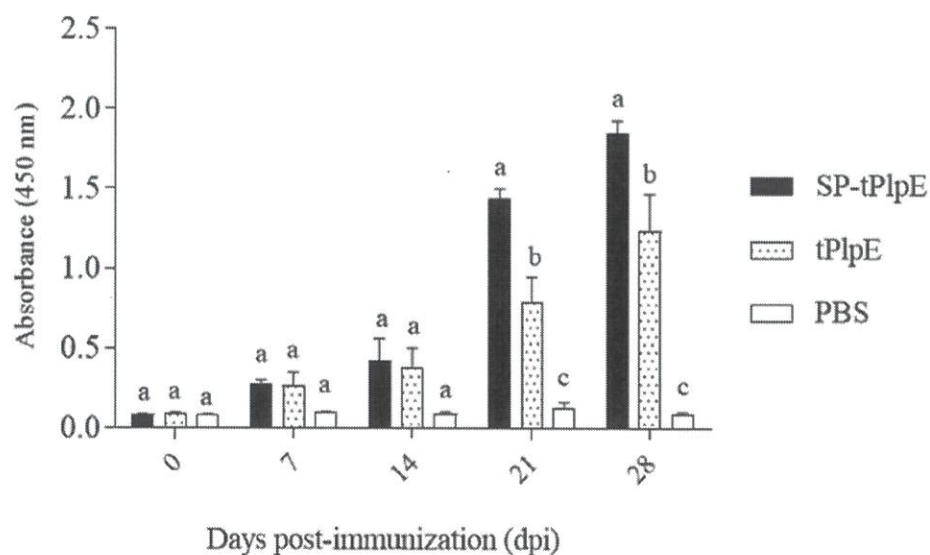
Signal Sequence enhance cytokine expression profile



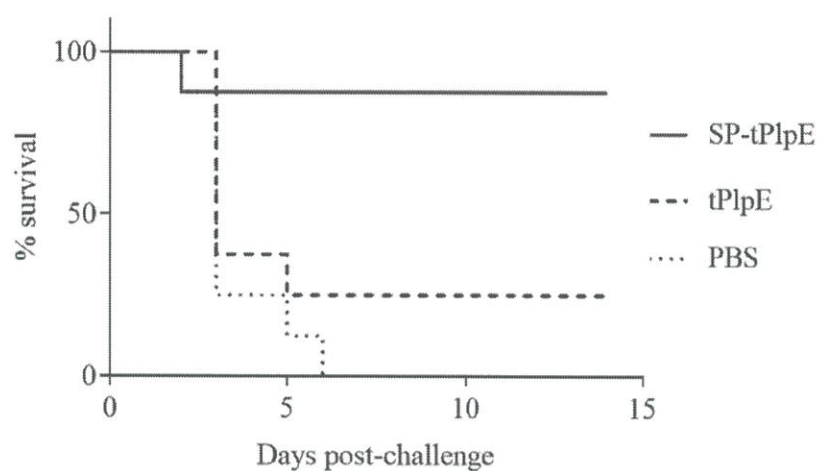
Signal Sequence Enhanced CD4⁺ T Cell Populations



Signal Sequence Enhanced Antibody Production



Signal peptide enhance survival in challenge Assay



Thank you for
your time

