Highly Pure, Multi-Epitopic Lipopeptide Vaccine Delivery System: Synthesis and Investigation

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Introduction

Vaccination is the most effective/cost-effective public health intervention

- Disease prevention
- Reduces health care costs
- Reduces lost work time due to sickness

Adapted from: Med Res Rev 1997;17:277

**Traditional Approaches**

**Killed Vaccines**
- e.g. Rabies vaccine

**Attenuated “Weakened” Vaccines**
- e.g. BCG vaccine (tuberculosis)

**Toxoids**
- e.g. Diphtheria vaccine

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**Infectious Agent** → **Culture Techniques** → **Inactivation** → **Selection of Avirulent Strains** → **Subunit Vaccines** → **e.g. DNA vaccines, virus like particles (VLPs), peptide vaccines**
Introduction

Subunit Vaccines

Contain the minimal microbial components necessary to stimulate an appropriate immune response

- Vaccines are administered to healthy individuals (normally children).
- These people are being asked to take a medication when they are well.
- Therefore adverse effects must be minimal.

Advantage

- Removing unnecessary components, reduces the risk of auto-immune diseases and adverse effects.
- Not infectious; No reversion to virulence.
- Can customise the vaccine components to tailor an appropriate immune response.

Problem

- Removing unnecessary components often removes danger signals.
- Need strong adjuvant (‘immune stimulating agent’).
- In the case of peptides:
  - Small molecular weight limits their capacity to elicit immune responses.
  - Peptides lack the T-helper epitopes required for efficacy in an outbred population.
The Lipid-Core Peptide (LCP) System

Poly-lysine
Multiple Antigen Peptide (MAP) System
( Carrier)
PNAS 1988;85:5409

Lipoamino acid
Liebigs Ann Chem 1990;(12):1175

Peptide (Antigen)

Peptide
α Lys

Peptide
α Lys

Peptide
ε

Peptide
ε

Lipid Core (Adjuvant)

* Mimics Pam₃Cys

Tetrahedron Lett 1993;34:3925

www.uq.edu.au
1. **Synthesize LCP Lipid Core**
   - Using stepwise solid-phase peptide synthesis

2. Synthesize Tetravalent MAP
   • Using stepwise solid-phase peptide synthesis
3. Synthesize Peptide Antigens
   • Using stepwise solid-phase peptide synthesis
3. **Cleave Peptide From Resin and Purify**
   - Cleave using hydrogen fluoride
   - Purify by gel filtration/HPLC

Peptide Antigen

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LCP-88/30-J8

C_{536}H_{918}N_{164}O_{166}S_{2}  

12380.16 g/mol

J8: QAEDK VKQSR EAKKQ VEKAL KQLED KVQ (28mer)

88/30: DNGKA IYERA RERAL QELGP C (21mer)

A/ 0.1% TFA/H_{2}O  
B/ 90% IPA/0.1% TFA/H_{2}O

Gradient: 0-100%B over 30min
Flowrate: 1mL/min
Detection: 214nm
Column: Vydac 214TP54 (5µm; 250 × 4.6mm)

t_R: 19.625 min
Subcutaneous Immunization

B10.BR (H-2^k) mice (n=10) 4-6 week old female
Prime: 30µg LCP-88/30-J8 either 1:1 in CFA or in 50µL PBS
Boost: 3µg in PBS, days 21, 28, 35, 42, & 49

Intra-peritoneal Challenge

400µL (1 x 10^5 CFU/mL 88/30 GAS)

Systemic IgG Antibody Titers (ELISA)
**LCP-88/30-J8**

**Systemic IgG Antibody Titers (Intranasal)**

- **Prime:** 30-60 µg LCP-88/30-J8 ± 10 µg cholera toxin B-subunit in 20 µL PBS
- **Boost:** days 7, 14, 21, 28, 35

**Intranasal Immunization**

B10.BR (H-2K) mice 4-6 week old female

**Oral Immunization**

Prime: 100 µg LCP-88/30-J8

±10 µg cholera toxin in 0.2M sodium bicarbonate

Boost: weekly intervals; 5 (with CT) or 7 (without CT)
Project Aims

Lipid Core Peptide System

Advantages:
• High antibody (IgG) titers against attached peptides
• Comparable with the highly toxic adjuvant complete Freund’s adjuvant (CFA)
• Potentially safe (non-toxic) for use in humans

Disadvantages:
• Difficult to purify
• Not suitable for use in human clinical trials

Project Aim:
• To develop a method to enable the synthesis of highly pure, easily characterized analogues of the lipid core peptide system

Techniques to be assessed:
• Solution- and solid-phase native chemical ligation
• Fragment condensation
Native Chemical Ligation (NCL)

- Formation of “Native” peptide bond

**C-terminal Peptide**
- Contains N-terminal Cysteine

**N-terminal Peptide**
- Contains C-terminal Thioester
  - Aqueous denaturing conditions
    - 6M Gdn.HCl
    - Urea
    - Phosphate buffer
  - Performed at pH 7-8
    - Minimal side reactions

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Curr Opin Biotech 1998;9:412
Based on:
J Org Chem 2000;65(12):3829

Resin:
Diaminodipropylamine (DAPDA) derivatized 4% crosslinked agarose beads (16µmol NH₂/mL; Pierce Biotechnology, Rockford IL)

SCAL Linker:

Boc-safety-catch acid labile linker (CSPS pharmaceuticals, San Diego CA)

In oxidised (SO) form:
Stable to TFA, HF, 50% piperidine, Pd(0)

In reduced (S) form:
Cleaved by 50% TFA

Reducing agent:
SiCl₄
Problems with Solid-Phase NCL

Problems:
- Poor solubility of lipid adjuvant in aqueous buffers
- Addition of organic solvents (e.g. TFE, MeCN, DMF, dioxane)
  - Solubilizes lipidic adjuvant
  - Ligation does not occur
- Need excess of thioester peptide to push ligation to completion (wasteful)
- Monitoring of ligation reactions and protecting group removals difficult
  - RP-HPLC provides some quantitative data
- Cleavage of product from the resin is problematic
- The resin is not completely stable to the conditions used for ligation and protecting group removal

Possible Solution to Solubility Issue:
- Use fragment condensation to couple lipid adjuvant to resin, then use NCL to ligate immunogenic peptides
**Fragment Condensation**

**Fragment Condensation:**
- Solubility in DMF of lipid adjuvant good.
- Coupling took over 24 hours

**Native Chemical Ligation:**
- Only 33% complete despite using 2eq thioester peptide
- Subsequent ligations worse

**Conclusion:**
- Difficult, expensive, and wasteful
- Use solution phase ligation
Solution-Phase NCL

**Problems:**
- Poor solubility of lipid adjuvant in aqueous buffers
- Ligation does not occur

Addition of organic solvents (e.g. TFE, MeCN, DMF, dioxane)
- Solubilizes lipidic adjuvant
- Ligation does not occur

LCP Lipid Core

Cys-C12-Gly-C12-Gly-NH₂

**Ligation**
- 0.1M phosphate buffer pH 7.5
- MESNA
- TCEP

Ac-Peptide Antigen Lys-Gly-S-Cys-C12-Gly-C12-Gly-C12-Gly-NH₂

Ac-Peptide Antigen Lys-Gly-Cys-C12-Gly-C12-Gly-C12-Gly-NH₂
NCL + SDS

LIGATION

1% (w/v) SDS
0.1M phosphate buffer pH 7.5
TCEP
MESNA

CLEAVAGE

TFA
Thioanisole
p-cresol
EDT
SiCl₄

MW 3350.18 g/mol
Synthesis of a Highly Pure LCP-Analogue

**THIOL EXCHANGE**
Mesna 0.1M Phosphate Buffer pH 7.5

Ac-[PL1]-Lys-Gly-C12-Gly-C12-C12-Gly-NH2

**LIQUATION**
1% (w/v) SDS
TCEP 0.1M Phosphate Buffer pH 7.5

Ac-[PL1]-Lys-Gly-C12-Gly-C12-C12-Gly-NH2

**THIOL EXCHANGE**
Mesna 0.1M Phosphate Buffer pH 7.6

Ac-[88/30]-Lys-Gly-Cys

**ACM DEPROTECTION**
i) I2, 1:1 AcOH-H2O
ii) 1M ascorbic acid
TCEP

Ac-[88/30]-Lys-Gly-Cys

**LIGATION**
1% (w/v) SDS
TCEP 0.1M Phosphate Buffer pH 7.6

Ac-[PL1]-Lys-Gly-C12-Gly-C12-C12-Gly-NH2

44.1%
Overall Yield

**RP-HPLC**
Solvent A: 0.1% TFA/H2O
Solvent B: 90% ACN/0.1%
TFA/H2O
Flowrate: 1mL/min
Column: Vydac C4 (214TP54; 300Å; 5µm,
4.6 x 250mm)
Gradient: 0% to 100% B over 30 min
Detection: 214nm
tR: 18.733 min
Purity: 97.7%
LCP-analogue 1

\[
Ac-[PL1]-Lys-Gly-C12-Gly-C12-C12-Gly-NH_2
\]

Ac-[88/30]-Lys-Gly-Cys

Ac-[J8]-Gly-Cys

J Med Chem 2006;49(21):6364

LCP-system

LCP-analogue 2

\[
Ac-[PL1]-Lys-Gly-NH_2
\]

Ac-[J8]-Lys-Gly-Cys

Ac-[88/30]-Lys-Gly-Cys

(Gly)_4

\[
C12-Gly-(C12)_2-Gly
\]

J Org Chem 2006;71(18):6846

1) THIOL EXCHANGE
MESNA
0.1M Phosphate Buffer pH 7.6

2) LIGATION
1% (w/v) SDS
TCEP
0.1M Phosphate Buffer pH 7.6

Ac-[88/30]-Gly-S-\text{Leu-NH}_2

Ac-[88/30]-Gly-S-\text{SO}_3^-\text{Na}^+

Ac-[88/30]-Gly-Cys

Ac-[88/30]-Gly-Lys

Ac-[88/30]-Gly-Lys-C12-Gly-C12-C12-Gly-NH_2

Ac-[88/30]-Gly-Lys-Cys

Ac-[88/30]-Gly-Cys

Product

MW 10990.49g/mol (exact 10983.76)

C_{42}H_{44}N_{12}O_{24}S_{4}
Subcutaneous Immunisation

Mice: 4-6 week old ♀ B10.BR (H-2<sup>k</sup>)
Immunised at the tail base
1°: 30µg in 50µL PBS or 1:1 CFA
Boosts: 3µg in PBS (days 21, 28, 35, 42, & 49)
Conclusions

• Demonstrated a method for the synthesis of highly pure, multi-epitopic, self-adjuvanting lipopeptide vaccines.
  – Required the use of SDS
• May prove useful for the synthesis of multi-epitopic vaccines against diseases caused by other microorganisms.
Acknowledgements

This research is proudly supported by the Queensland Government's Growing the Smart State PhD Funding Program. The State of Queensland accepts no responsibility for decisions or actions resulting from any information supplied. The views and information contained in the research do not necessarily represent the views or opinions of the Queensland Government and carry no endorsement by the Queensland Government.