Liposomal mucosal vaccine delivery system: immunogenicity, inflammatory response and protection from group A streptococcus challenge.

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Mucosal vaccine – An unmet clinical need

- The majority of infections begin from mucosal surfaces including the upper respiratory tract (URT). For many of these infections there are currently no effective solutions.

- There is a clear unmet clinical need for better vaccine strategies to combat diseases caused by URT infection.
URT pathogen - Group A Streptococcus (GAS)

- Group A streptococcus (GAS) is a Gram-positive bacteria that occurs in chains or in pairs of cells.
- GAS infection and associated diseases result in over 500,000 deaths each year globally.
Current GAS vaccines in development

- The cell surface M protein is a major virulence determinant of GAS.

- GAS vaccines can be broadly divided into M protein–based and non–M protein–based vaccines.

<p>| TABLE 1. GAS Vaccine Antigen Candidates (Adapted From Steer et al(^5)) |
|--------------------------------------------------|--------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Vaccine Class</th>
<th>Vaccine Antigen</th>
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</thead>
<tbody>
<tr>
<td>M protein: N-terminal region</td>
<td>6-valent, 26-valent and 30-valent N-terminal</td>
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<tr>
<td>M protein: Conserved region</td>
<td>Whole C-repeat conserved region</td>
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<tr>
<td></td>
<td>Minimal epitope J8/J14/p145</td>
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<td></td>
<td>C-repeat epitope (StreptInCor vaccine)</td>
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<tr>
<td></td>
<td>C-repeat epitopes</td>
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<tr>
<td>Non–M protein vaccine candidates</td>
<td>GAS carbohydrate</td>
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<td></td>
<td>GAS C5a peptidase</td>
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<tr>
<td></td>
<td>Fibronectin-binding protein</td>
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<tr>
<td></td>
<td>Streptococcal protective antigen</td>
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<tr>
<td></td>
<td>Serum opacity factor</td>
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<tr>
<td></td>
<td>Streptococcal pyrogenic exotoxin B (extracellular cysteine protease)</td>
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<tr>
<td></td>
<td>Streptococcal pyrogenic exotoxin C</td>
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<tr>
<td></td>
<td>Streptococcal pili (T antigen)</td>
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<tr>
<td></td>
<td>Serine protease (SpyCEP)</td>
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<tr>
<td></td>
<td>Serine esterase (Sse)</td>
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<td></td>
<td>GAS 40</td>
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<tr>
<td></td>
<td>Nine common antigens</td>
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<tr>
<td></td>
<td>G-related α2-macroglobulin binding protein</td>
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<tr>
<td></td>
<td>Metal transporter of streptococcus (MtsA)</td>
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<tr>
<td></td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td></td>
<td>Lipoproteins</td>
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</table>

- GAS vaccines that have entered or are nearing clinical trials are the N-terminal M protein based multivalent vaccines (26-valent and 30-valent vaccines) and conserved M protein vaccines.
Opsonic antibodies directed against the N terminus of the M protein are responsible for serotypic immunity (more than 180 serotypes exist).

Conserved C-terminal of the protein is identical in 70% of group A streptococci isolates.

The optimal candidate might consist of conserved determinant to overcome serotypic variability.

Our vaccine strategy is to raise antibodies against protective B cell epitopes from the conserved C3 region of the M protein.

Target GAS B Cell Epitope (J8)

QAEDKVQKSREAKKQVEKALKQLEDKVQ
Challenges associated with peptide vaccines

REQUIREMENTS

- Immune response linked to particular HLA (MHC) alleles, so some peptides not universally effective at inducing protective immunity
  - Need Helper T-cell epitopes
  - Current dogma is chemical conjugation to carrier protein.

- Immune response relatively weak so effective delivery system/adjuvants required.

- Mucosal vaccine development is restricted by lack of mucosal adjuvants compatible for use in humans for the delivery of peptides.
Liposomal mucosal vaccine delivery system

- A liposome is a small vesicle made out of the same material as a cell membrane (phospholipids).

- Liposomes can be filled or surface loaded with drugs and used to deliver drugs for diseases.

- Advantage of liposomes is that it is biocompatible.
Our liposomal GAS vaccine candidate: J8-Lipo-DT

DT = Diphtheria toxoid providing CD4+ T cell epitope for antibody response

• J8 QAEDKVKQ SREAKKQVEKALKQLE DKVQ
J8-Lipo-DT formulation

Liposome synthesis:
1. Mixing phospholipids, cholesterol & palmitic acid modified J8 in chloroform.
2. Remove solvent by evaporation.
3. Hydration with PBS containing DT to form liposomes
4. Liposomes separated from nonentrapped DT by centrifugation at 14000 × g for 10

- Encapsulation Efficiency (EE) calculated by Amino Acid Analysis of DT content within liposomes.

<table>
<thead>
<tr>
<th>Amount Of DT used for rehydration (µg)</th>
<th>Amount DT within liposomes (after centrifugation and removal of supernatant)</th>
<th>Entrapment efficiency (EE) %</th>
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</thead>
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<tr>
<td>150 µg</td>
<td>87.4 µg</td>
<td>58.3%</td>
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</table>
J8-Lipo-DT characterization

- Utilised flow cytometry to identify components of J8-Lipo-DT.

- 95% of DT is associated with Liposomes containing J8.

* Diptheria toxoid (conjugated to CF405S succinimidyl ester)
J8-Lipo-DT characterization: size of formulation

- Determined using a Nanosizer (Dynamic light scattering or DLS)
- DLS revealed size of liposomes to be 1756 nm (Std Dev = 100.3 nm).
- The majority of particles had a narrow molecular-weight distribution (polydispersity index of 0.238).
J8-Lipo-DT immunogenicity: mucosal IgA & systemic IgG

- Mice administered 30 μg of J8-Lipo-DT (primary + 2 boosts).
- Comparable immune response to mice administered J8 conjugated to DT and co-administered with animal restricted mucosal adjuvant CTB (J8-DT+CTB).
J8-Lipo-DT efficacy: protection against GAS challenge

- J8-Lipo-DT immunized mice had significantly less colonisation of the URT post-challenge.

- To measure the intensity of infection of the URT, throat swabs were monitored.

- Nasal associated lymphoid tissue (NALT) in mice is structurally and functionally analogous to human tonsils (reservoir for GAS).

- J8-Lipo-DT immunized mice had significantly lower bacterial load to PBS group in NALT.
J8-Lipo-DT mechanism of action: pro-inflammatory cytokine response

- J8-Lipo-DT immunized mice secrete antigen specific pro-inflammatory cytokines.

- Human and murine IL-6 plays a critical role in B cell terminal differentiation, proliferation and secretion of IgA in mucosal sites.

- IL-6 is known to be a signal for neutrophil production and neutrophils are essential for IgA-mediated opsonization of GAS.
J8-Lipo-DT mechanism of action: immune response in IL-6 KO mice

- J8-specific IgA response not IL-6 mediated.

- IL6 KO mice had significantly higher total streptococcal tissue bioburden (pharynx, NALT and lungs) post-challenge in comparison to wildtype mice.

- Shows the importance of inflammatory cellular responses in J8-Lipo-DT-mediated immunity and a likely explanation for the difference in protection against GAS in the tissues following J8-Lipo-DT and J8-DT/CTB vaccination.
J8-Lipo-DT mechanism of action: human DC maturation

- J8-Lipo-DT mediated expression of cell-surface activation and maturation markers on human plasmacytoid dendritic cells (CD123+ pDCs).

- Human pDCs readily phagocytose and process antigens entrapped in particulate delivery systems.

- pDCs have been identified in the blood, spleen, lymph nodes and mucosal sites including tonsils.

- J8-Lipo-DT significantly increased maturation marker CD83 and CD80/CD86 co-stimulatory molecules.
J8-Lipo-DT mechanism of action: human DC pro-inflammatory cytokine response

- J8-Lipo-DT induced substantial production of various levels of pro-inflammatory cytokines including IL-6, IL-8.

- IgA specific immunity against GAS requires the presence of neutrophils and IL-8 has a key role in the recruitment and activation of neutrophils.

- Mechanisms that are fundamental to conferring immunity in humans to GAS infection can be mediated using the liposome platform.
**J8-Lipo-DT mechanism of action: size-dependent immunogenicity**

- Immunise with nano to micro sized J8-Lipo-DT to see effect on immunogenicity.
- J8-Lipo-DT in larger sizes (823 nm & 1756 nm) induced significant IgA titer.
Mechanism of action: incorporation of other GAS antigens

- Hyper virulent GAS strains are characterized by up-regulated virulence factors including Streptococcus pyogenes cell envelope protease (SpyCEP).

- A vaccine was thus constructed using J8 together with another conserved peptide (from the bacterial IL-8 protease, SpyCEP) that can prevent infection with highly pathogenic mutant GAS (which up-regulate SpyCEP).

- The inclusion of a SpyCEP epitope (S2) alongside J8 in a single liposome construct was evaluated.
Mechanism of action: incorporation of other GAS antigens

- Liposome platform technology induces immune response against multiple epitopes from Group A Streptococcus.
Mechanism of action: protection against hyper virulent GAS challenge

- J8/S2-Lipo-DT immunized mice had significantly less colonisation of the URT post-challenge.

- Evidence that protection against the highly virulent GAS required the induction of antibodies capable of neutralizing two complementary virulence factors (M protein and SpyCEP).
Mechanism of action: DT provides T cell help required for S2-specific immune response

- DT conjugated to S2 (S2-DT+CTB) or encapsulated within liposomes (S2-Lipo-DT) induce an immune response.

- Spleen cell proliferation assay undertaken with mice immunized with S2+DT emulsified in complete Freund’s adjuvant (CFA).

- Cells were pulsed post 72h of antigenic stimulation with $^3$H-Thymidine and proliferation measured.
Mechanism of action: freeze-dried liposomes requires Trehalose for immunogenicity

- Lyophilized powder form negates cold-chain storage and stability issues of liposomes.

- Freeze-drying of J8-Lipo-DT resulted in formulations that are stable enough to be resuspended.

- Immunogenicity of freeze-dried liposome is largely determined by addition of trehalose.
Mechanism of action: incorporation of epitopes from multiple pathogens (Multi-vax)

- Multi-vax is a **universal vaccine against multiple URT pathogens** by incorporating subunit conserved antigens to broadly protect against all or most strains.

**Influenza A** (Fan J *et al.* Vaccine. 2004;22(23-24):2993-3003).

*DT = Diphtheria toxoid providing CD4+ T cell epitope for antibody response*
Multi-vax proof of concept: Immune response against multiple epitopes

- Multi-vax induces immunity against epitopes from multiple URT pathogens (Influenza A, Influenza B and Group A Streptococcus).
Summary

- Our study provides important mechanistic insights into how liposomal vaccine delivery systems can collectively induce the desired mucosal immune responses to combat GAS infection.

- Olymvax Biopharmaceuticals has agreed to fund further and major pre-clinical development of J8-Lipo-DT and to license the technology for clinical development.

- The strategy reported here is relevant to the development of subunit mucosal vaccines against other pathogenic organisms.
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