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

Mehfuz Zaman, Victoria Ozberk, Jennifer R. Reiman, Emma Langshaw, Michael R Batzloff, Manisha Pandey & Michael F Good.

Mucosal vaccine – An unmet clinical need

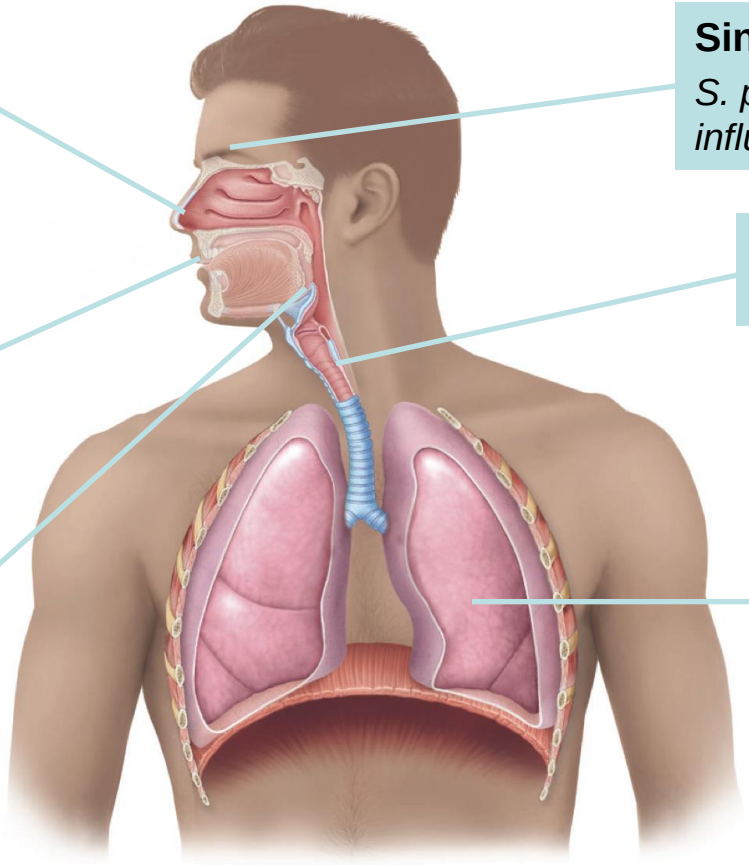
- The majority of infections begin from mucosal surfaces including the upper respiratory tract (URT). For many of these infections there are currently no effective solutions.

Common Cold
Rhinoviruses, Coronaviruses, Adenoviruses

Dental caries
S. Mutans

Stomatitis
Herpes Simplex Virus

Pharyngitis
Adenovirus, Herpes Simplex virus, Group A Streptococcus



Sinusitis
S. pneumoniae, H. influenzae

Whooping Cough
Bordetella pertussis

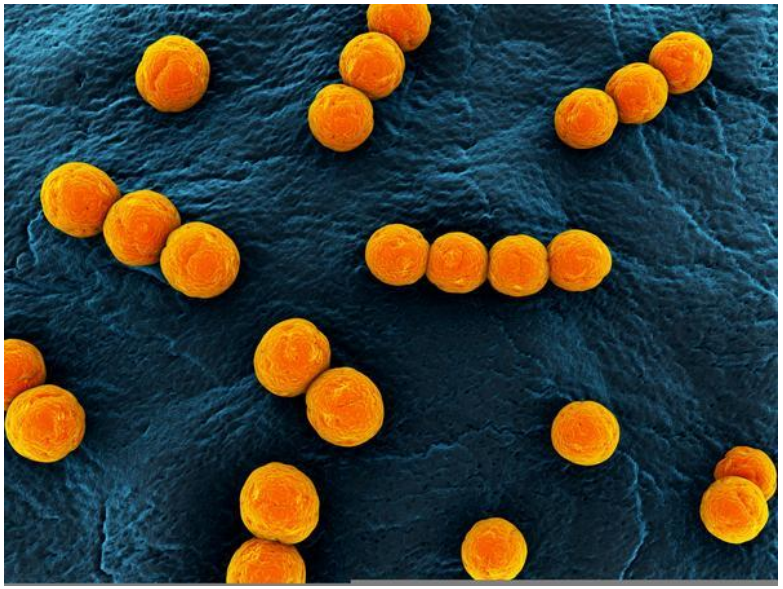
Bronchitis
Parainfluenza viruses, Respiratory Suncytial virus, Influenza virus, *M. pneumoniae, C. pneumoniae*

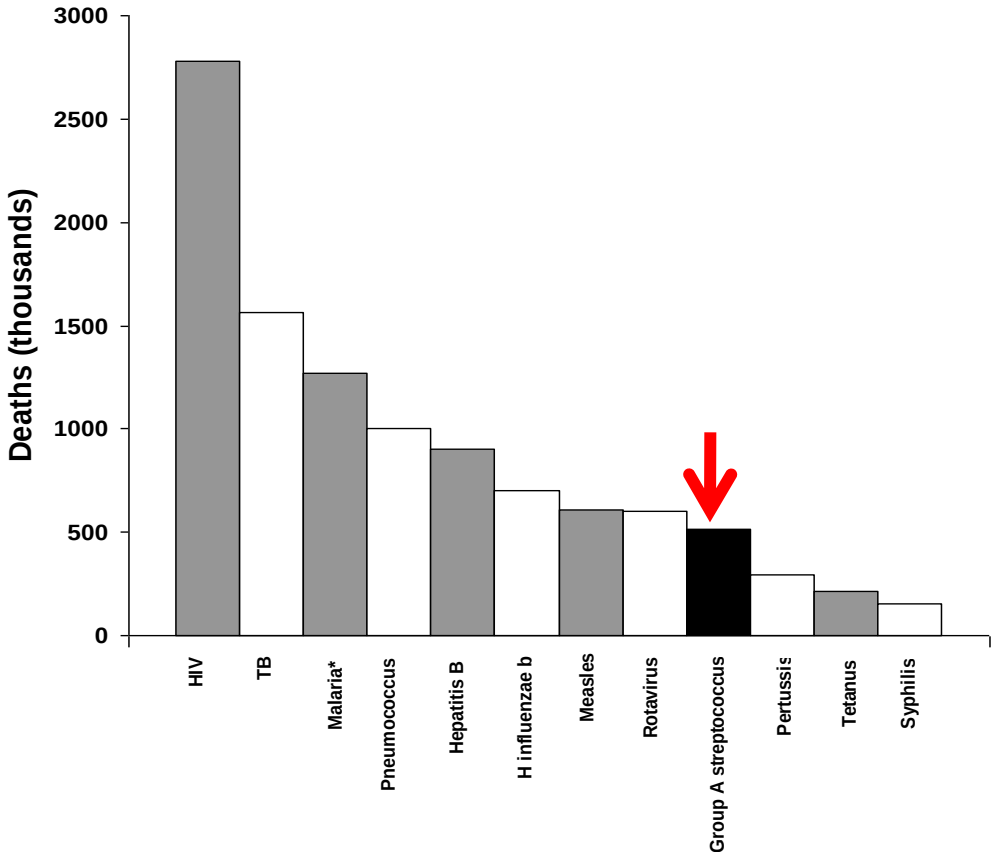
- **There is a clear unmet clinical need for better vaccine strategies to combat diseases caused by URT infection.**

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.

TABLE 1. GAS Vaccine Antigen Candidates (Adapted From Steer et al[5])

Vaccine Class	Vaccine Antigen
M protein: N-terminal region	6-valent, 26-valent and 30-valent N-terminal
M protein: Conserved region	Whole C-repeat conserved region
	Minimal epitope J8/J14/p145
	C-repeat epitope (StreptInCor vaccine)
	C-repeat epitopes
Non–M protein vaccine candidates	GAS carbohydrate
	GAS C5a peptidase
	Fibronectin-binding protein
	Streptococcal protective antigen
	Serum opacity factor
	Streptococcal pyrogenic exotoxin B (extracellular cysteine protease)
	Streptococcal pyrogenic exotoxin C
	Streptococcal pili (T antigen)
	Serine protease (SpyCEP)
	Serine esterase (Sse)
	GAS 40
	Nine common antigens
	G-related α2-macroglobulin binding protein
	Metal transporter of streptococcus (MtsA)
	Superoxide dismutase
	Lipoproteins

- **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.**

Our GAS vaccine strategy: minimal conserved B cell epitope from the M protein

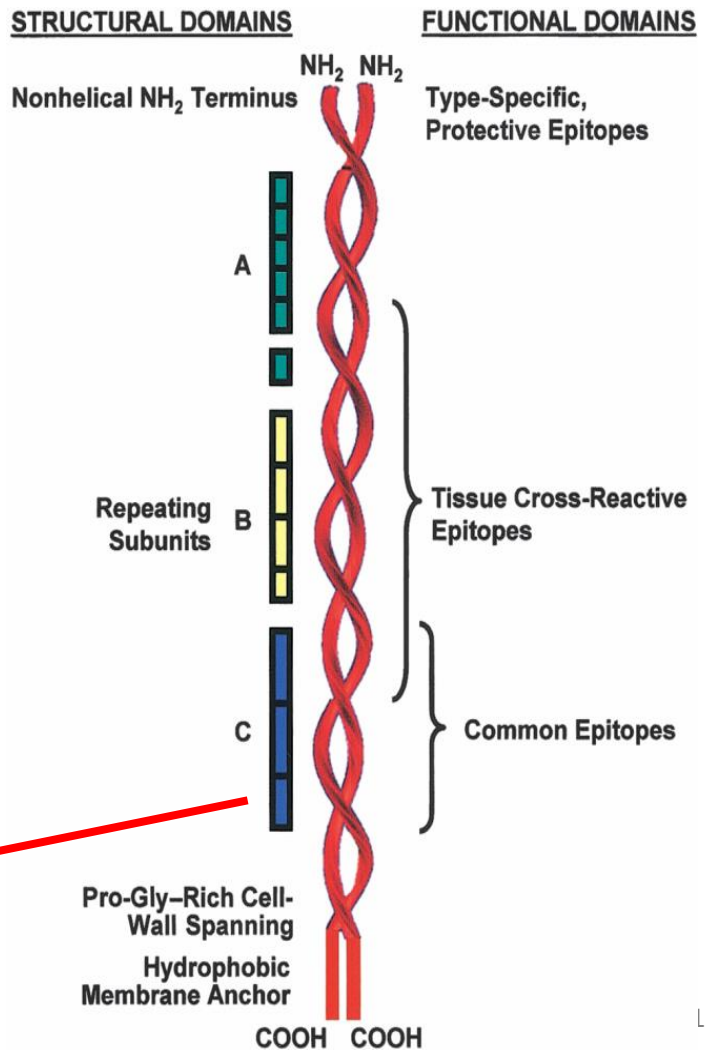
- Opsonic antibodies directed against the N terminus of the M protein are responsible for serotypic immunity (more than 180 serotypes exist).
- Conserved C-terminal of the protein is identical in 70% of group A streptococci isolates.
- The optimal candidate might consist of conserved determinant to overcome 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)

QAEDKVKQ**SREAKKQVEKAL**KQLEDKVQ

Schematic representation of GAS M proteins indicating the major structural and functional domains.

•Bisno AL, Rubin FA, et al. *Clin Infect Dis*. 2005;41(8):1150.



Challenges associated with peptide vaccines

REQUIREMENTS

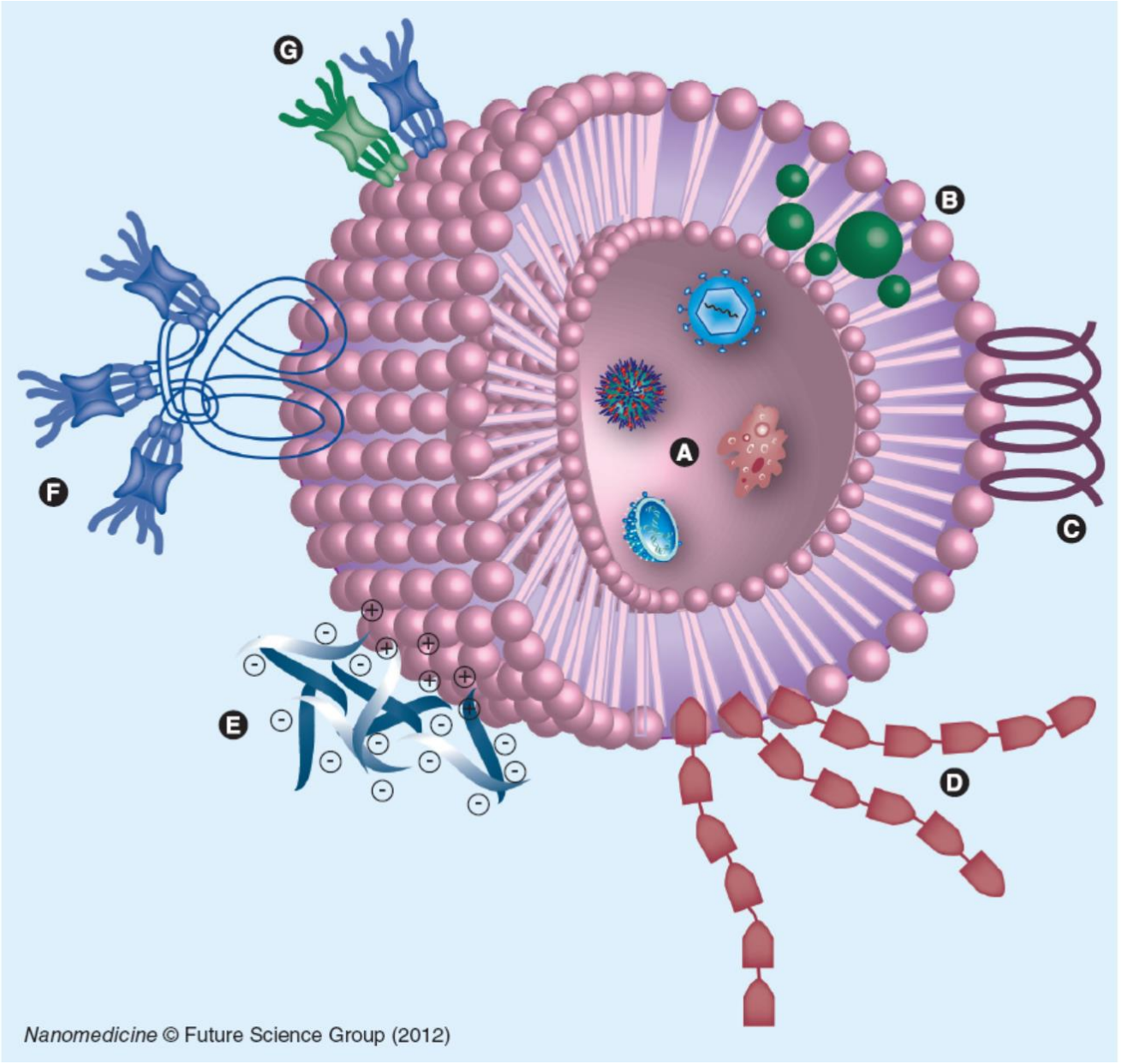
- Immune response linked to particular HLA (MHC) alleles, so some peptides not universally effective at inducing protective immunity
  - Need Helper T-cell epitopes
  - Current dogma is chemical conjugation to carrier protein.

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

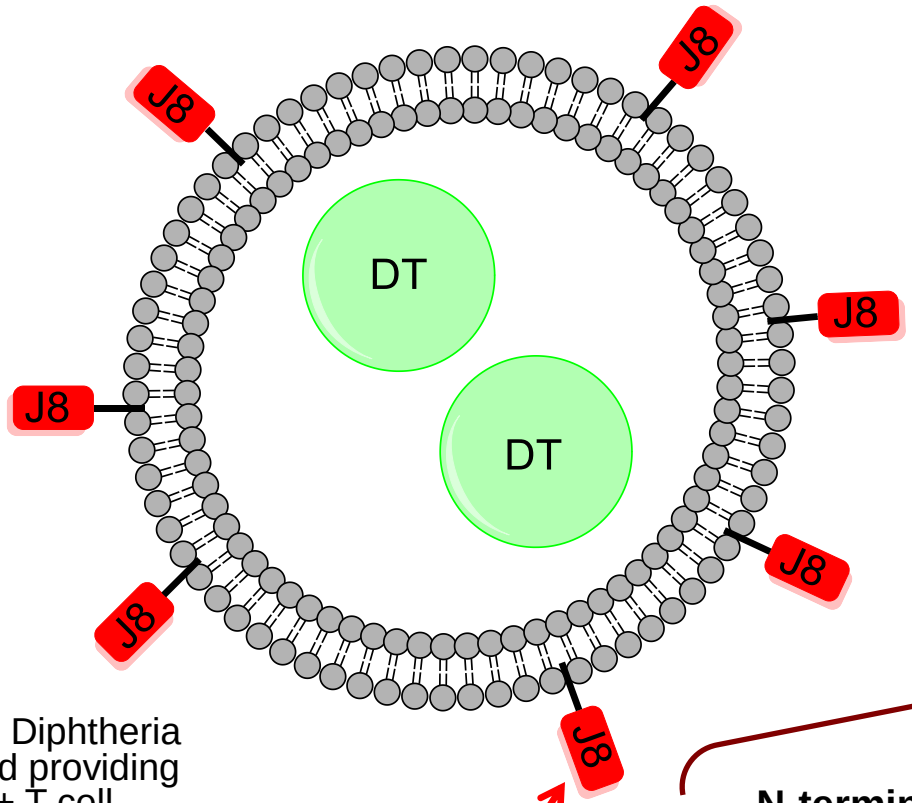
- **Mucosal vaccine development is restricted by lack of mucosal adjuvants compatible for use in humans for the delivery of peptides**.

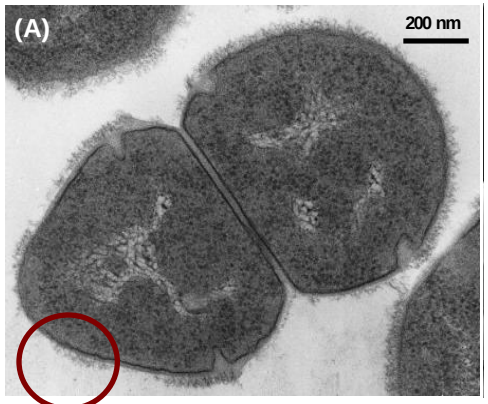
Liposomal mucosal vaccine delivery system

- 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

GAS

N-terminus **C-terminus**

Trans-membrane

A1 2 3 4 5 B1 2 3 4 C1 C2 C3 D1 D2

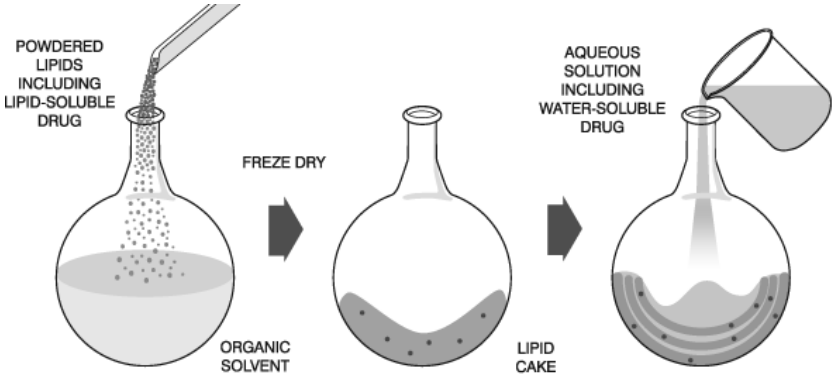
M protein

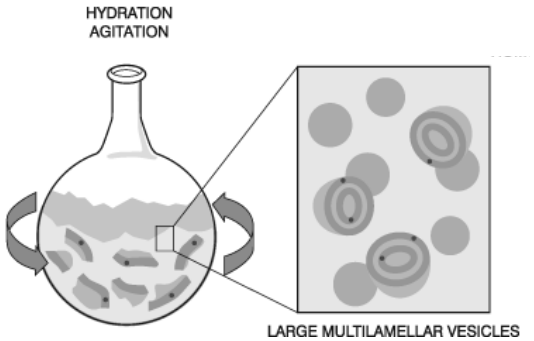
• J8 QAEDKVKQ**SREAKKQVEKALK**QLEDKVQ

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**



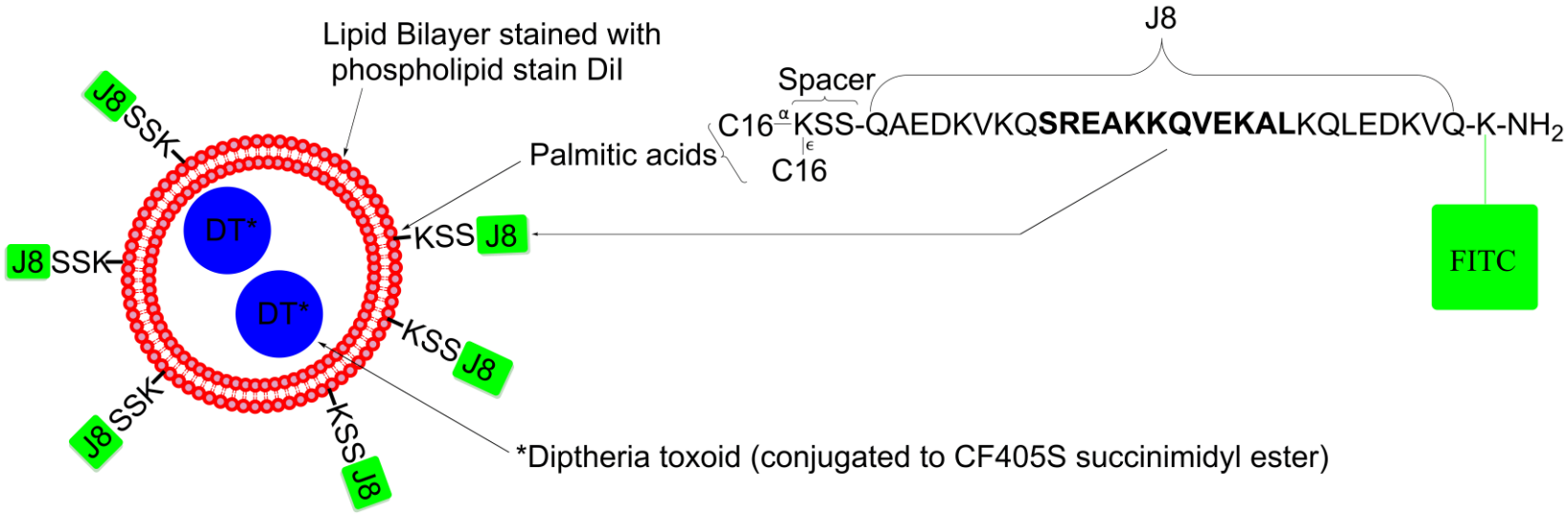


- Encapsulation Efficiency (EE) calculated by Amino Acid Analysis of DT content within liposomes.

Amount Of DT used for rehydration (µg)	Amount DT within liposomes (after centrifugation and removal of supernatant)	Entrapment efficiency (EE) %
150 µg	**87.4 µg**	58.3%

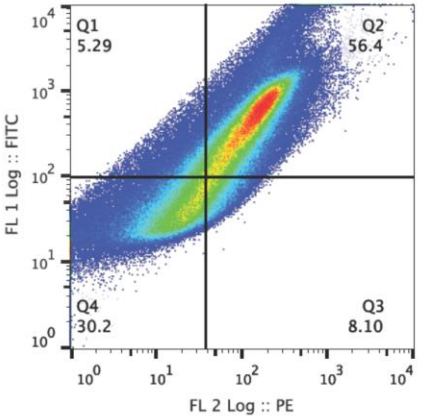
J8-Lipo-DT characterization

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

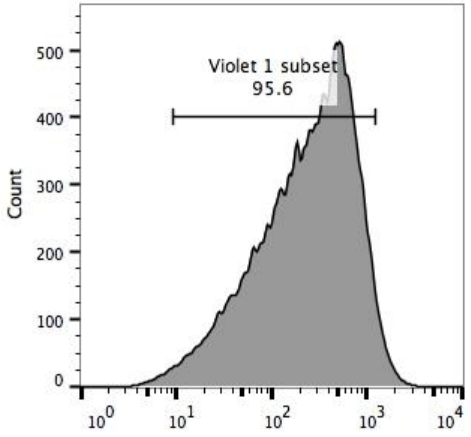


- **95% of DT is associated with Liposomes containing J8**.

Liposomes + J8
(DiI & FITC +ve)

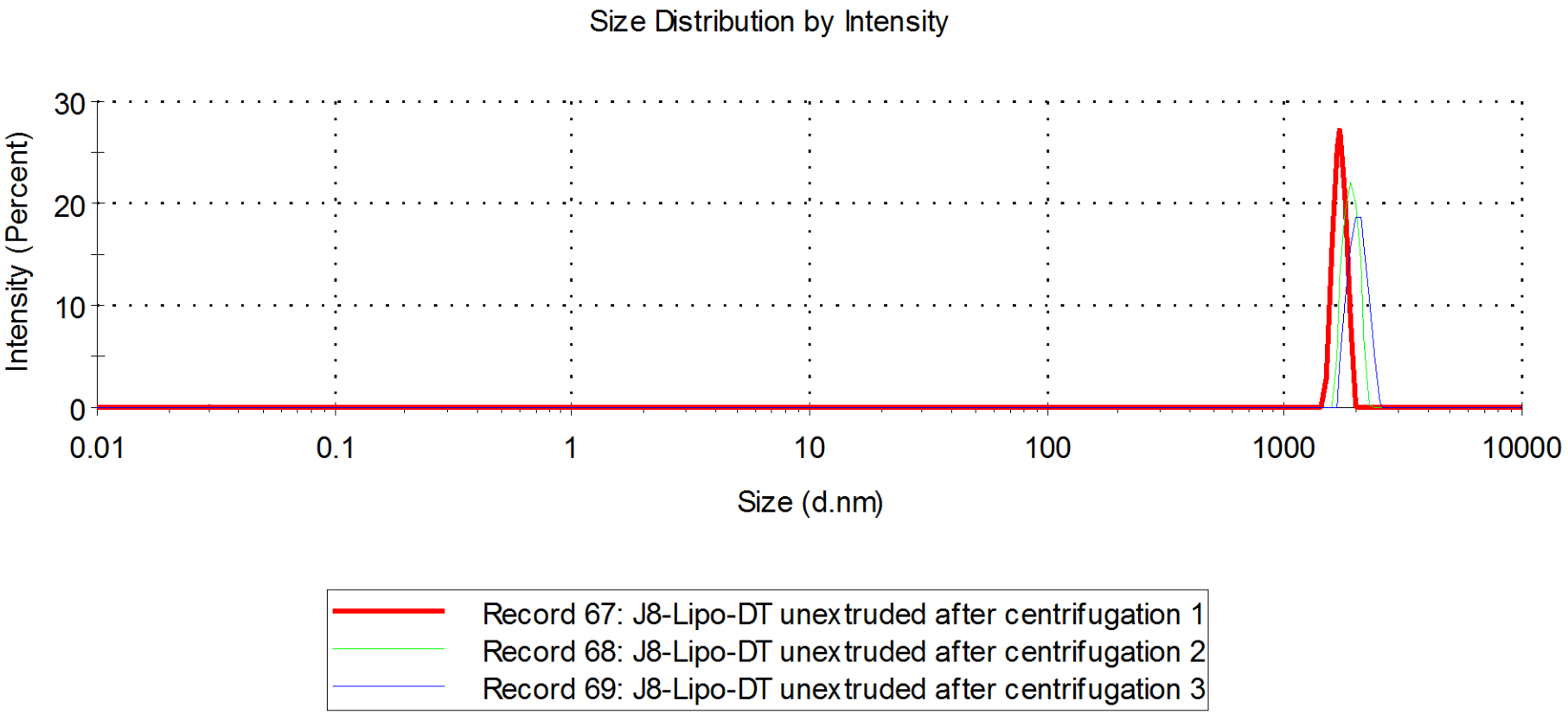


Liposomes + J8 + DT
(DiI, FITC & CF405s +ve)



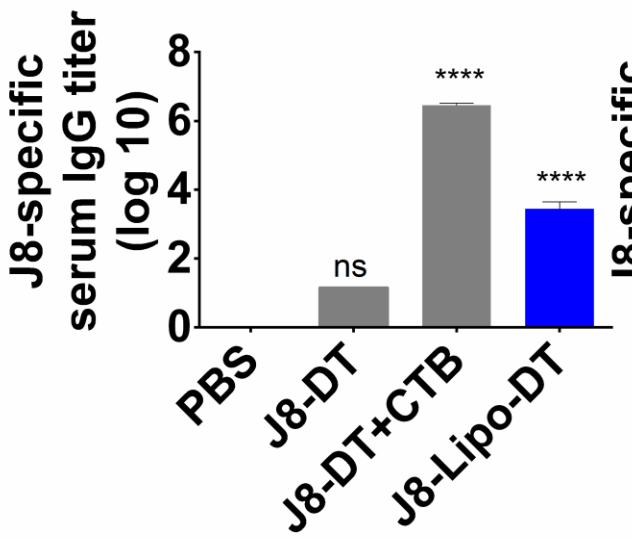
J8-Lipo-DT characterization: size of formulation

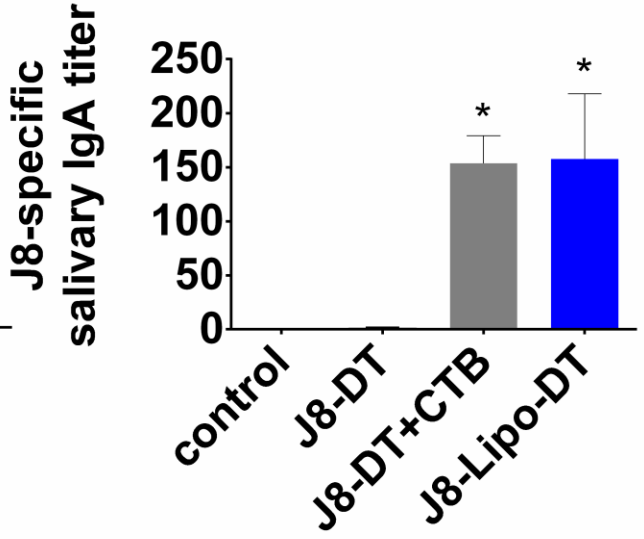
- Determined using a Nanosizer (Dynamic light scattering or DLS)
- DLS revealed size of liposomes to be 1756 nm (Std Dev = 100.3 nm).
- The majority of particles had a narrow molecular-weight distribution (polydispersity index of 0.238).

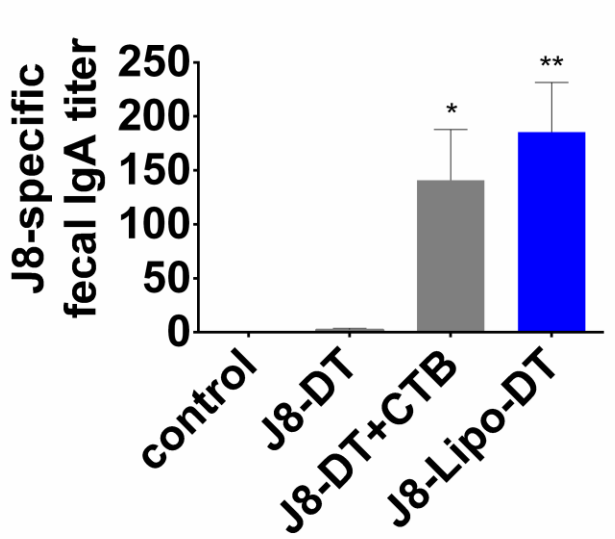


J8-Lipo-DT immunogenicity: mucosal IgA & systemic IgG

- 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).

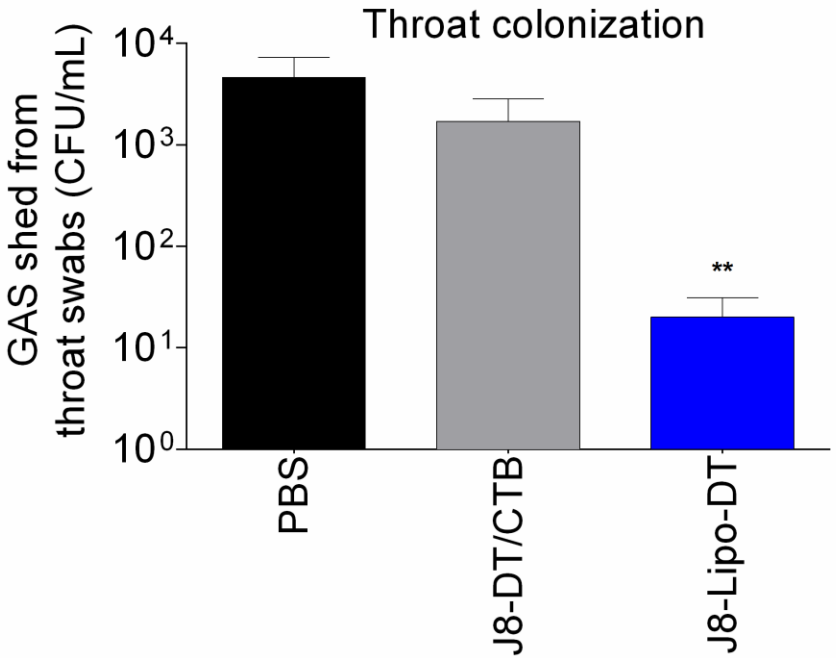


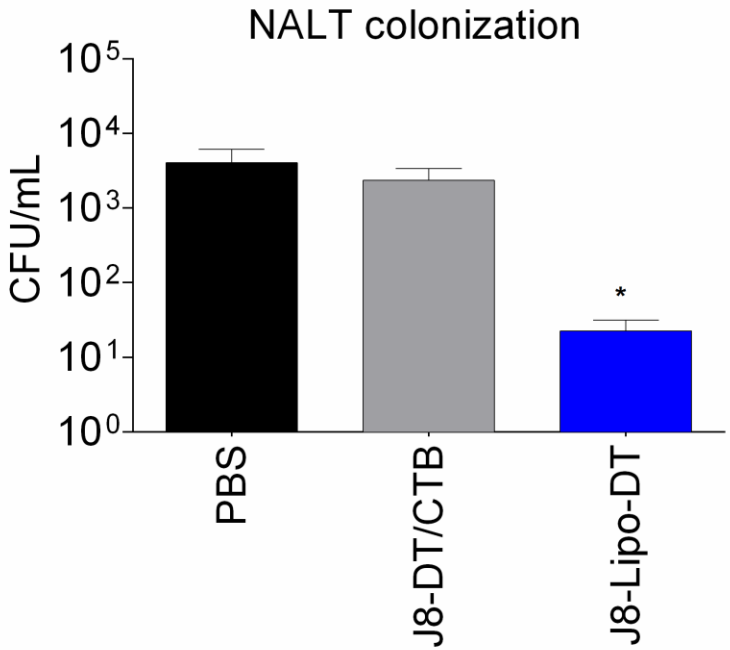




J8-Lipo-DT efficacy: protection against GAS challenge

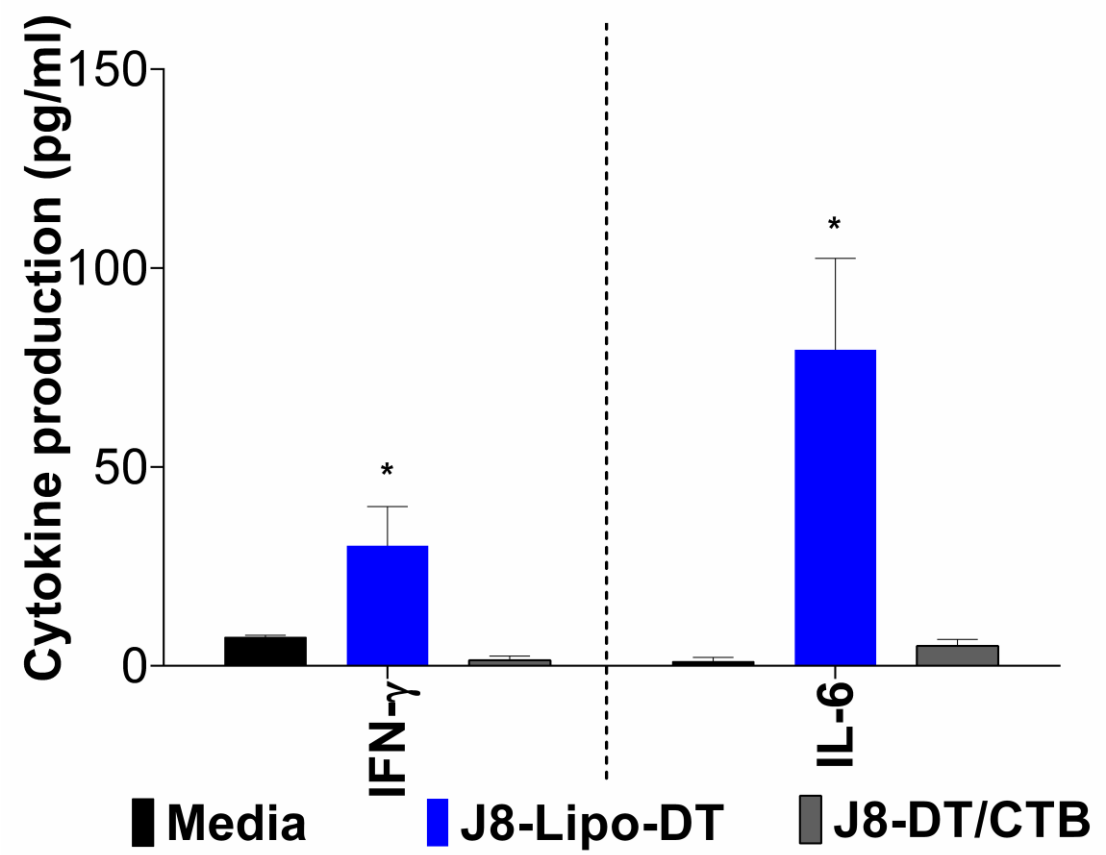
- J8-Lipo-DT immunized mice had significantly less colonisation of the URT post-challenge.
- To measure the intensity of infection of the URT, throat swabs were monitored.
- Nasal associated lymphoid tissue (NALT) in mice is structurally and functionally analogous to human tonsils (reservoir for GAS).
- J8-Lipo-DT immunized mice had significantly lower bacterial load to PBS group in NALT.





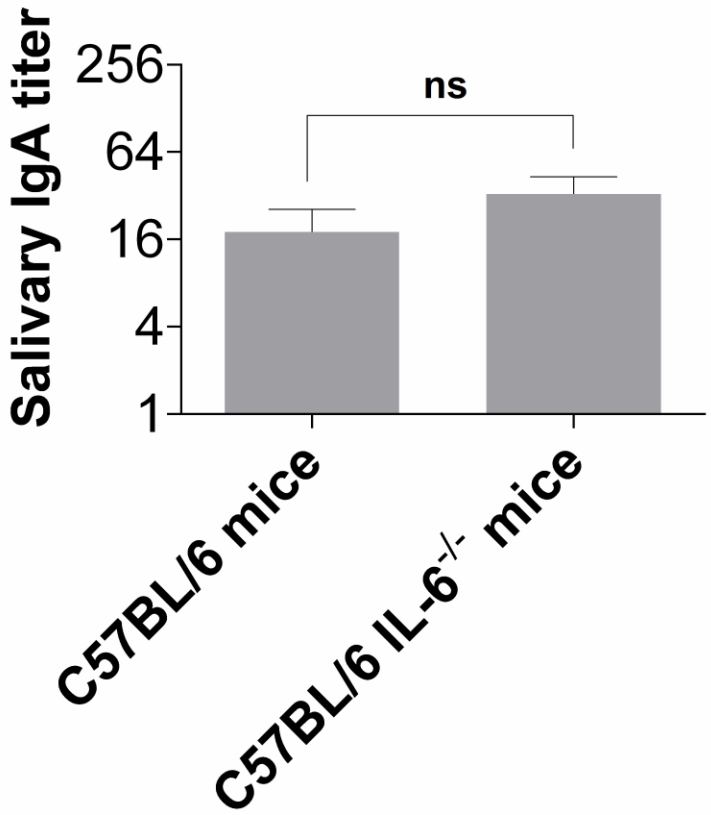
J8-Lipo-DT mechanism of action: pro-inflammatory cytokine response

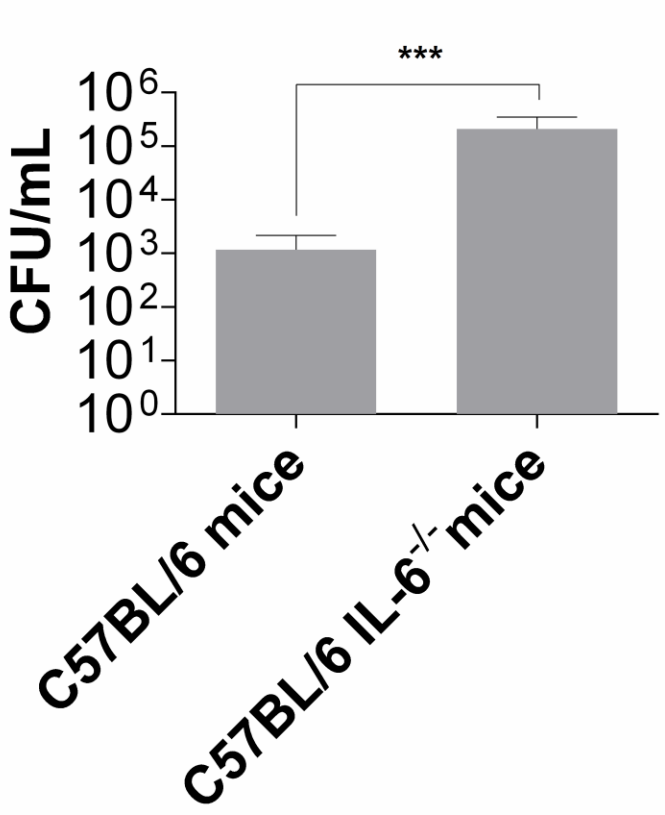
- J8-Lipo-DT immunized mice secrete antigen specific pro-inflammatory cytokines.
- Human and murine IL-6 plays a critical role in B cell terminal differentiation, proliferation and secretion of IgA in mucosal sites.
- IL-6 is known to be a signal for neutrophil production and neutrophils are essential for IgA-mediated opsonization of GAS.



J8-Lipo-DT mechanism of action: immune response in IL-6 KO mice

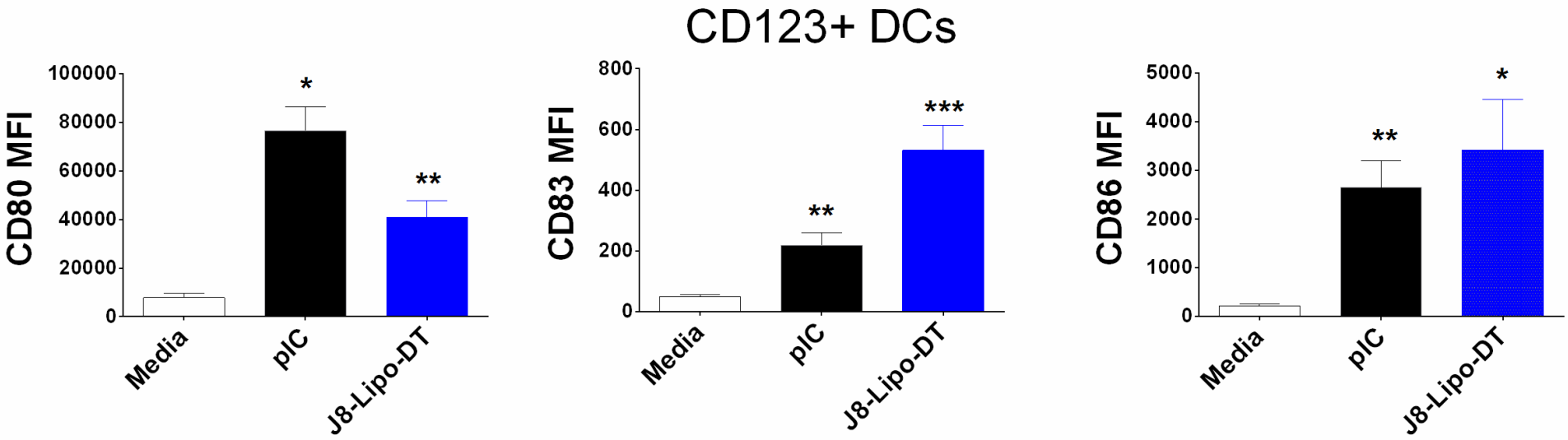
- J8-specific IgA response not IL-6 mediated.
- IL6 KO mice had significantly higher total streptococcal tissue bioburden (pharynx, NALT and lungs) post-challenge in comparison to wildtype mice.
- Shows the importance of inflammatory cellular responses in J8-Lipo-DT-mediated immunity and a likely explanation for the difference in protection against GAS in the tissues following J8-Lipo-DT and J8-DT/CTB vaccination





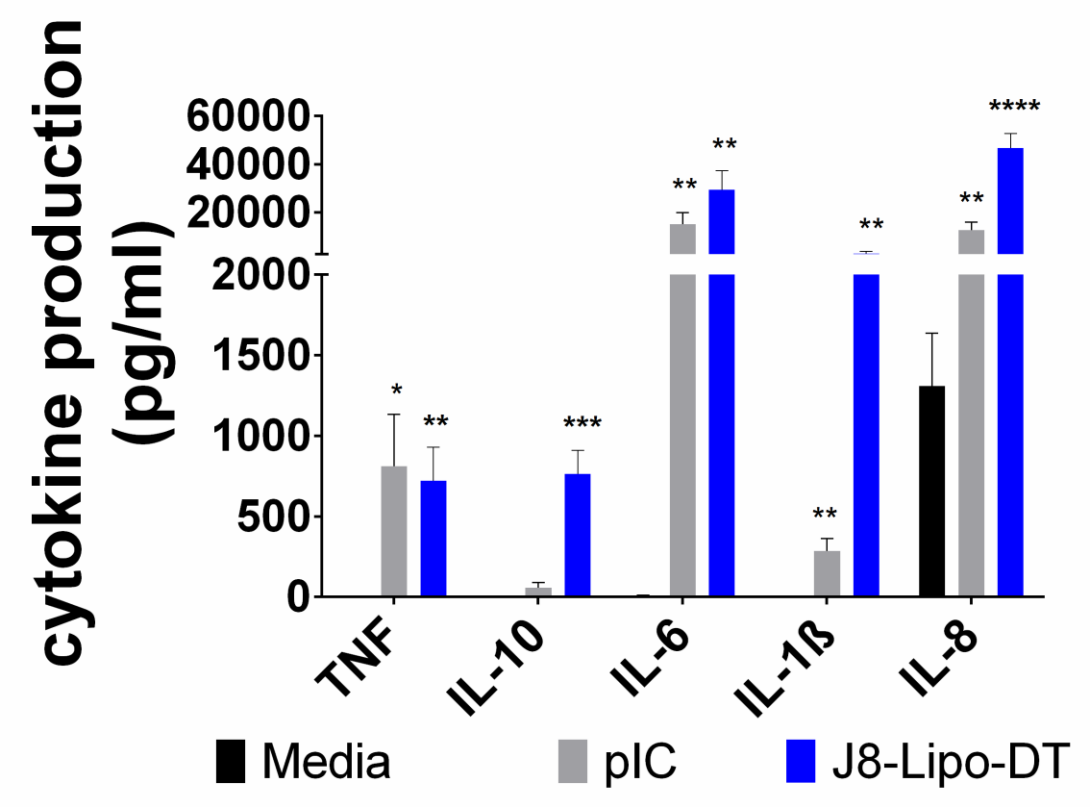
J8-Lipo-DT mechanism of action: human DC maturation

- 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.



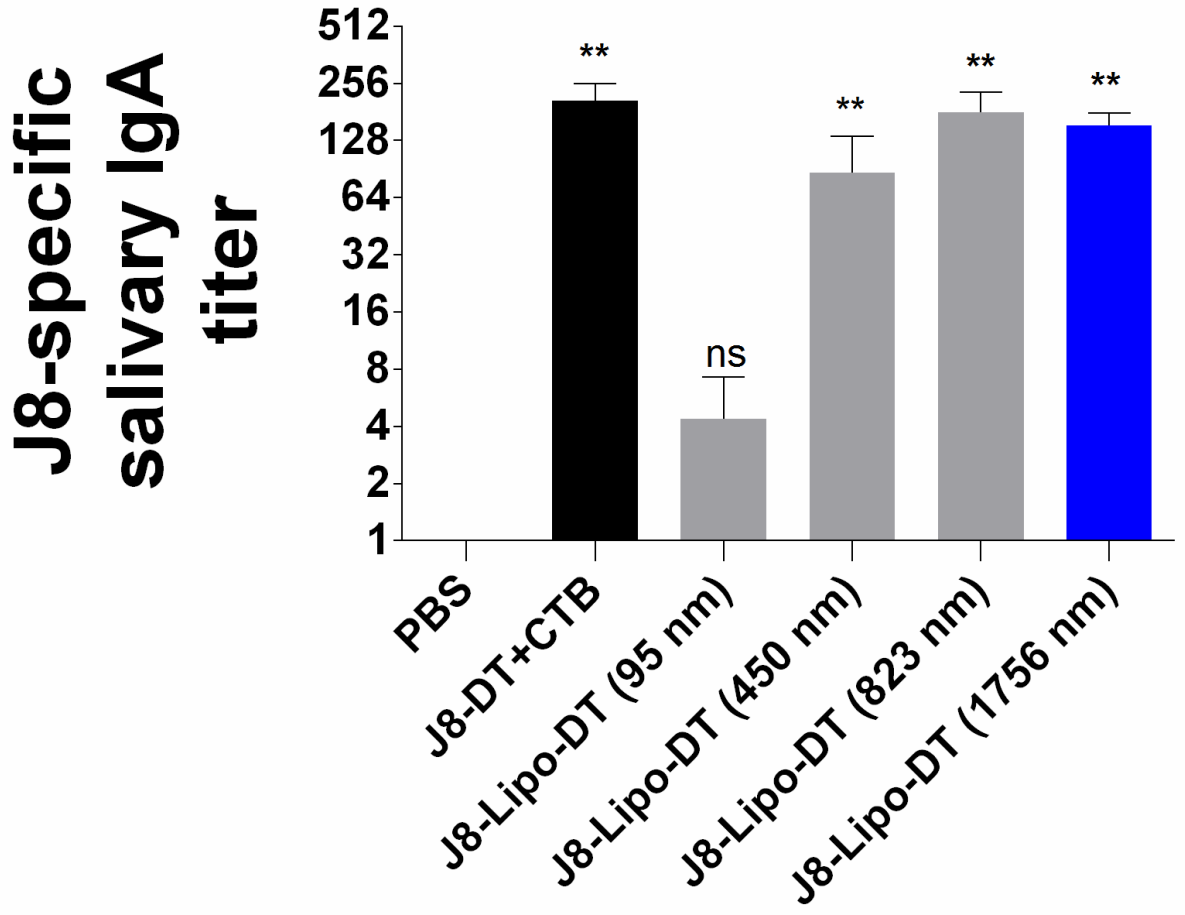
J8-Lipo-DT mechanism of action: human DC pro-inflammatory cytokine response

- 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



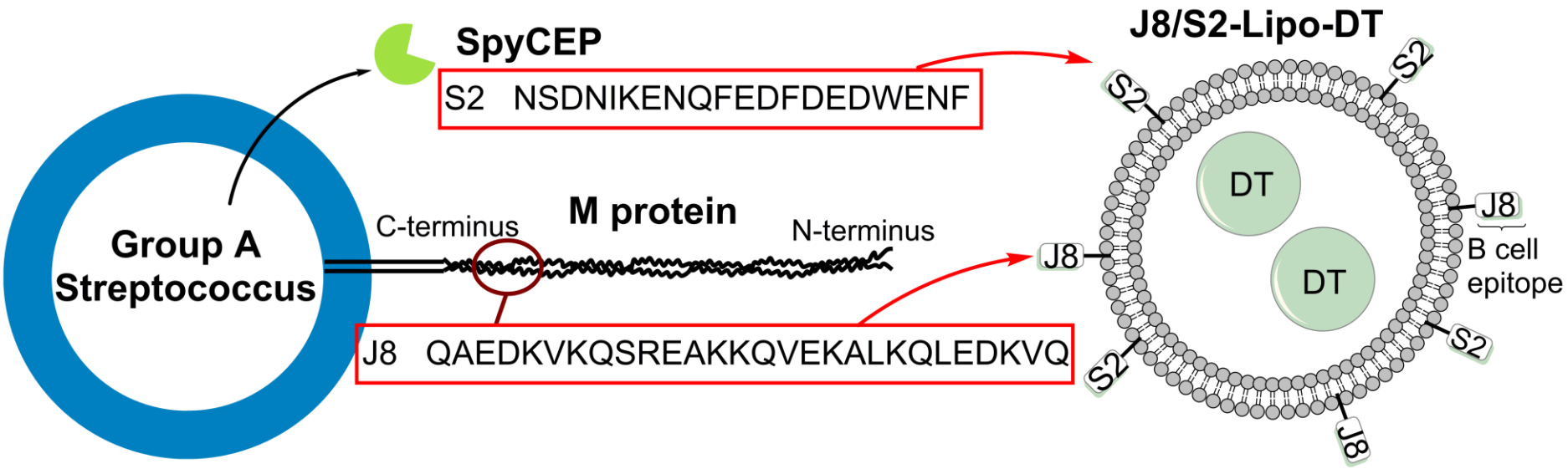
J8-Lipo-DT mechanism of action: size-dependent immunogenicity

- 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

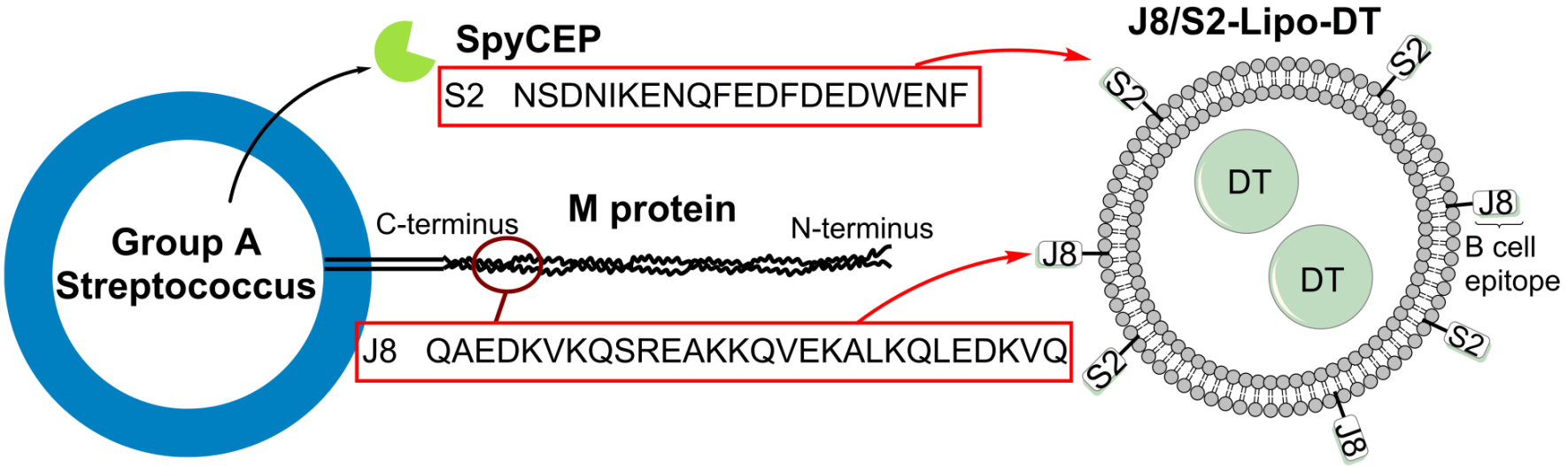
- Hyper virulent GAS strains are characterized by up-regulated virulence factors including Streptococcus pyogenes cell envelope protease (SpyCEP).
- A vaccine was thus constructed using J8 together with another conserved peptide (from the bacterial IL-8 protease, SpyCEP) that can prevent infection with highly pathogenic mutant GAS (which up-regulate SpyCEP).
- The inclusion of a SpyCEP epitope (S2) alongside J8 in a single liposome construct was evaluated.

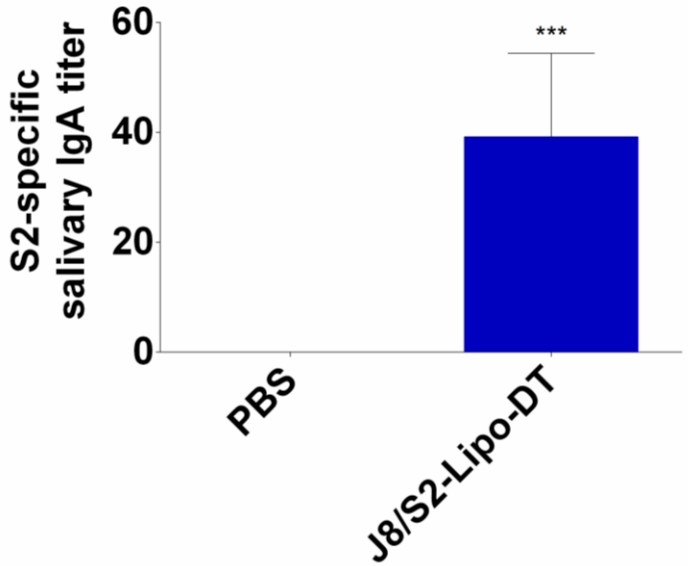


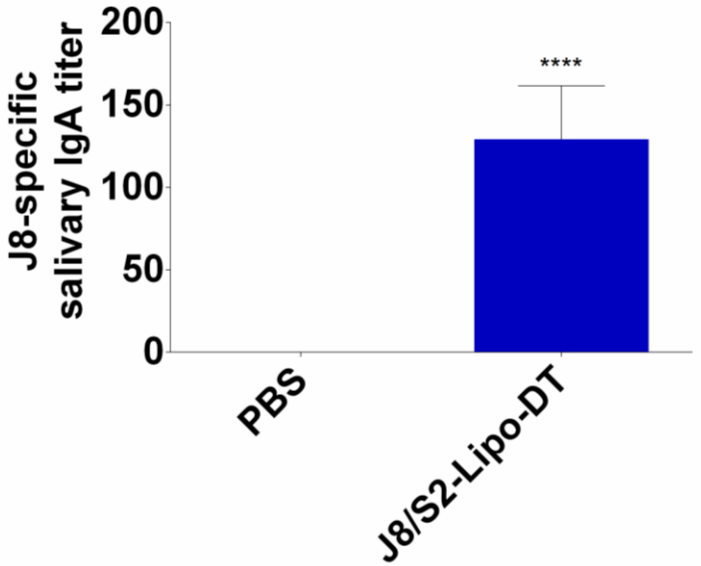
Pandey M *et al.* J Immunol. 2015 15;194(12):5915-25

Mechanism of action: incorporation of other GAS antigens

- Liposome platform technology induces immune response against multiple epitopes from Group A Streptococcus.



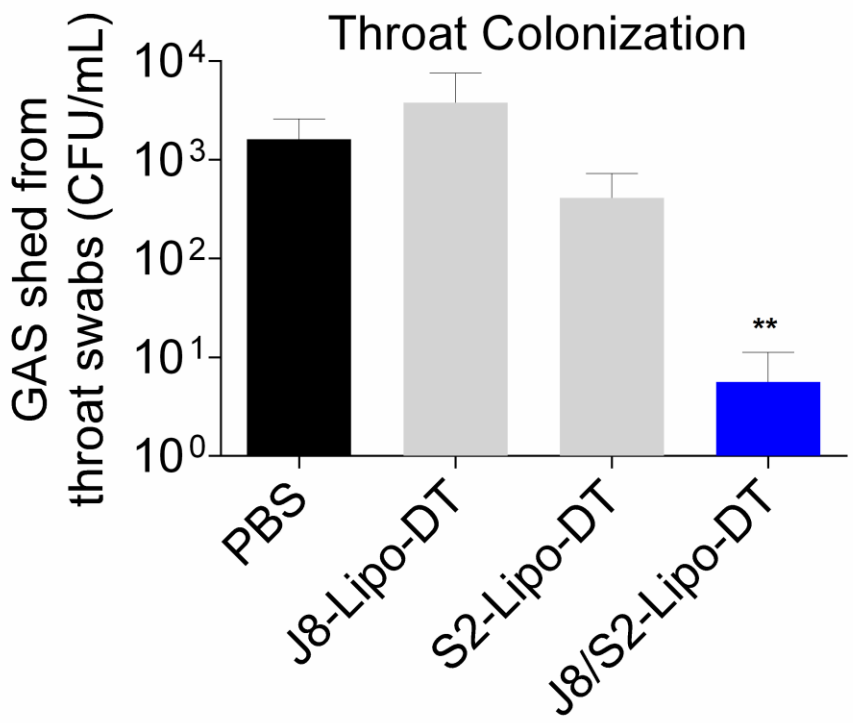


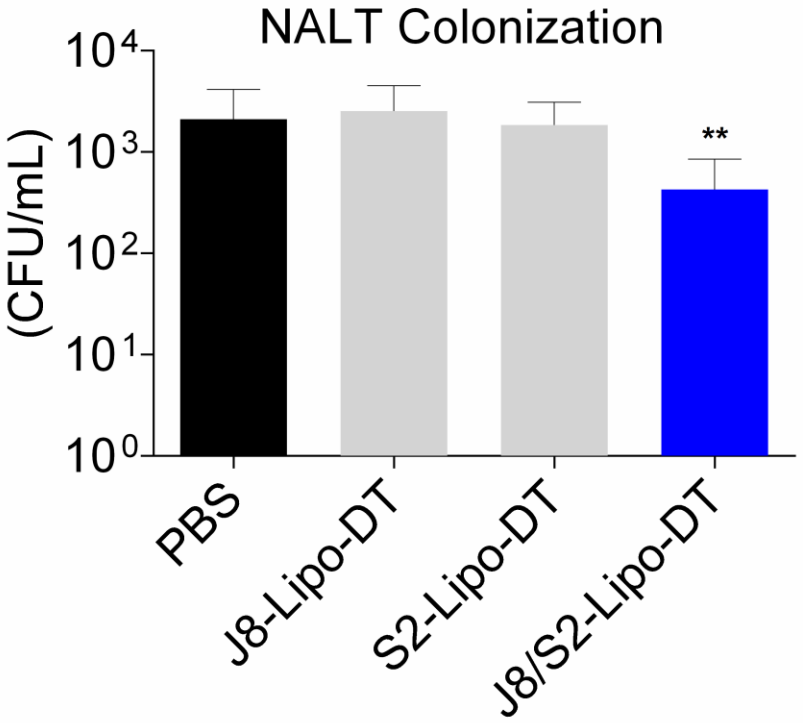


Mechanism of action: protection against hyper virulent GAS challenge

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

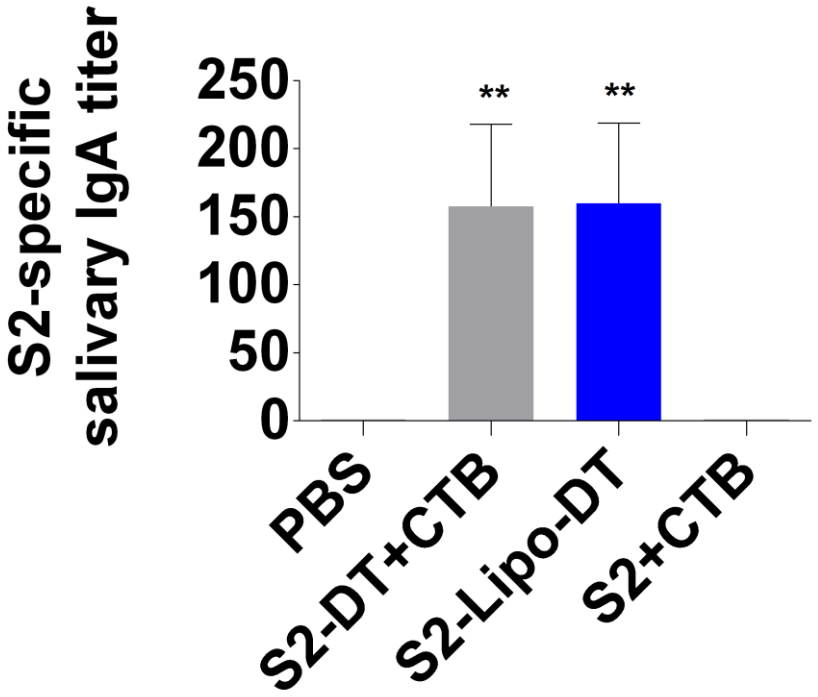
- Evidence that protection against the highly virulent GAS required the induction of antibodies capable of neutralizing two complementary virulence factors (M protein and SpyCEP).

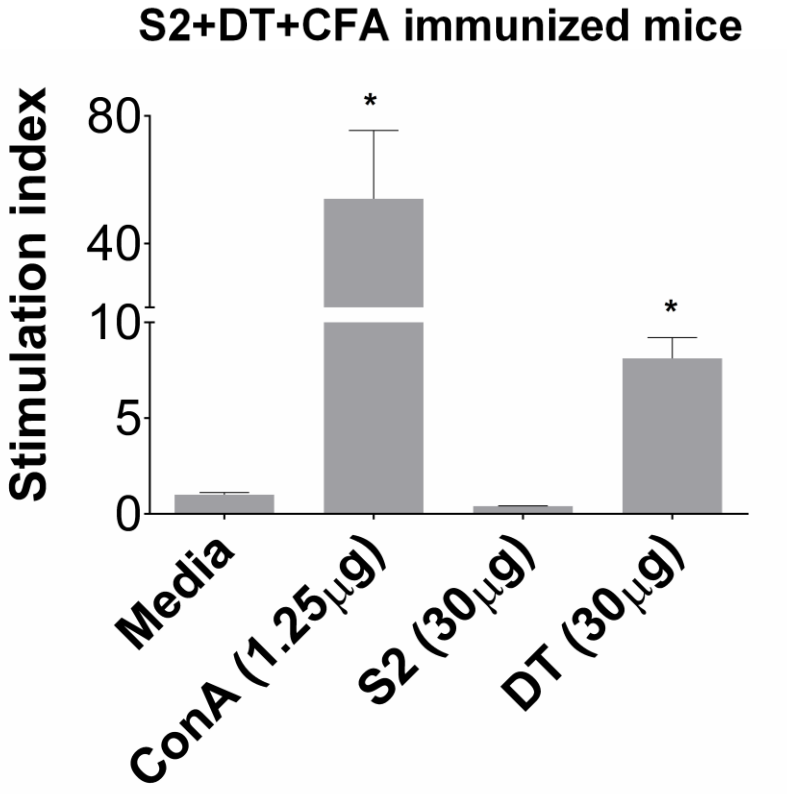




Mechanism of action: DT provides T cell help required for S2-specific immune response

- 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.

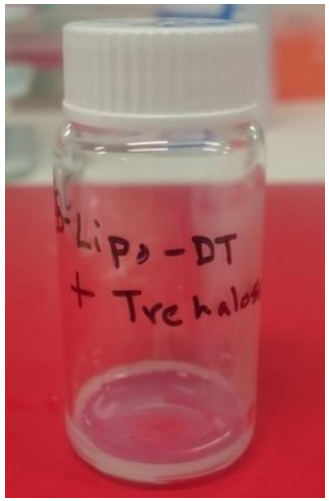


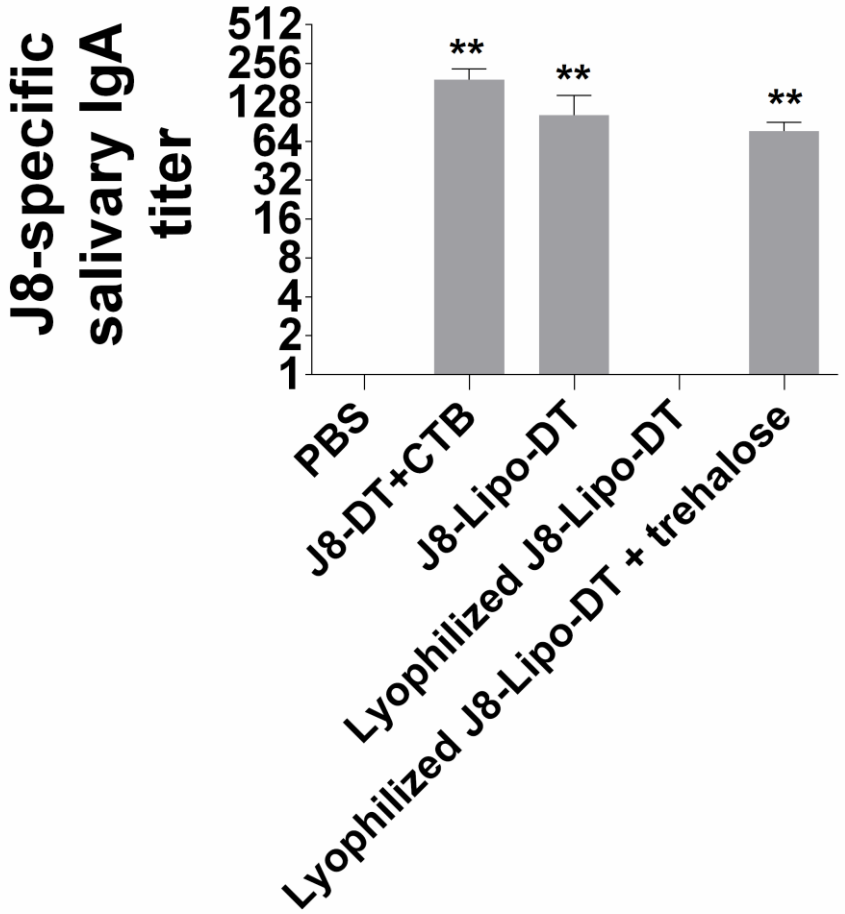


Mechanism of action: freeze-dried liposomes requires Trehalose for immunogenicity

- 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.

Freshly made
J8-Lipo-DT





Freeze-dried
J8-Lipo-DT+Trehalose

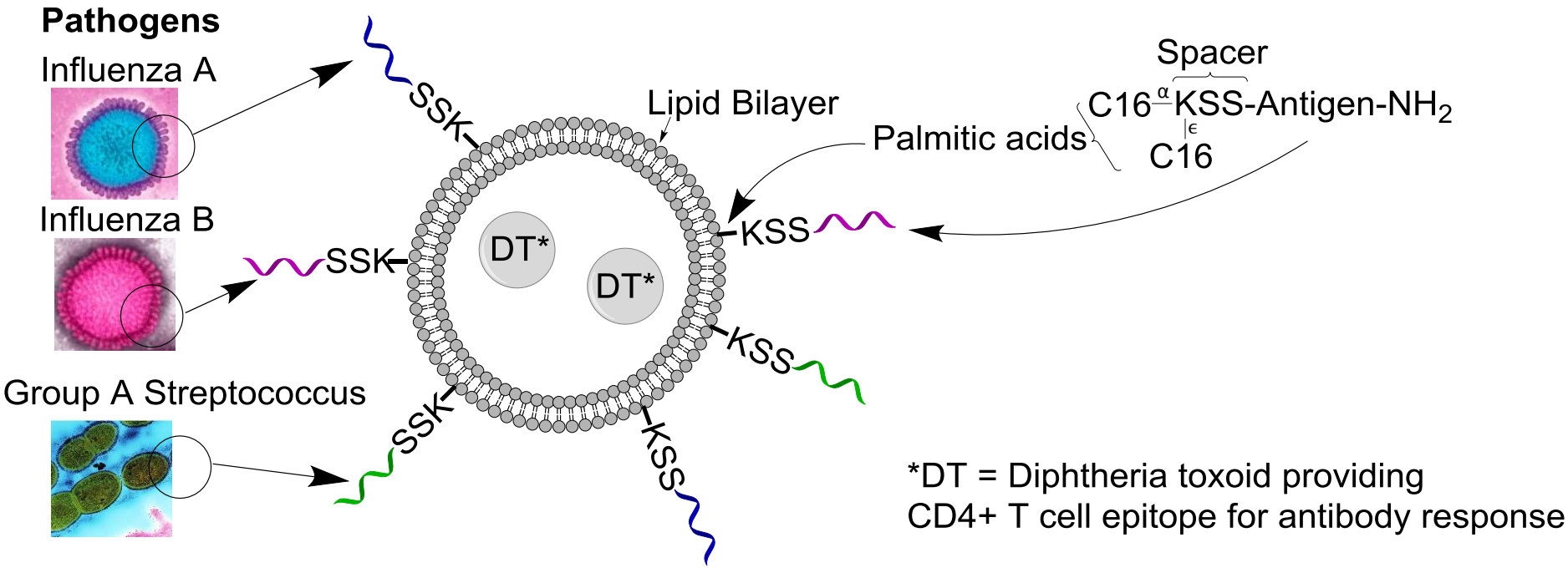
Mechanism of action: incorporation of epitopes from multiple pathogens (Multi-vax)

- 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).

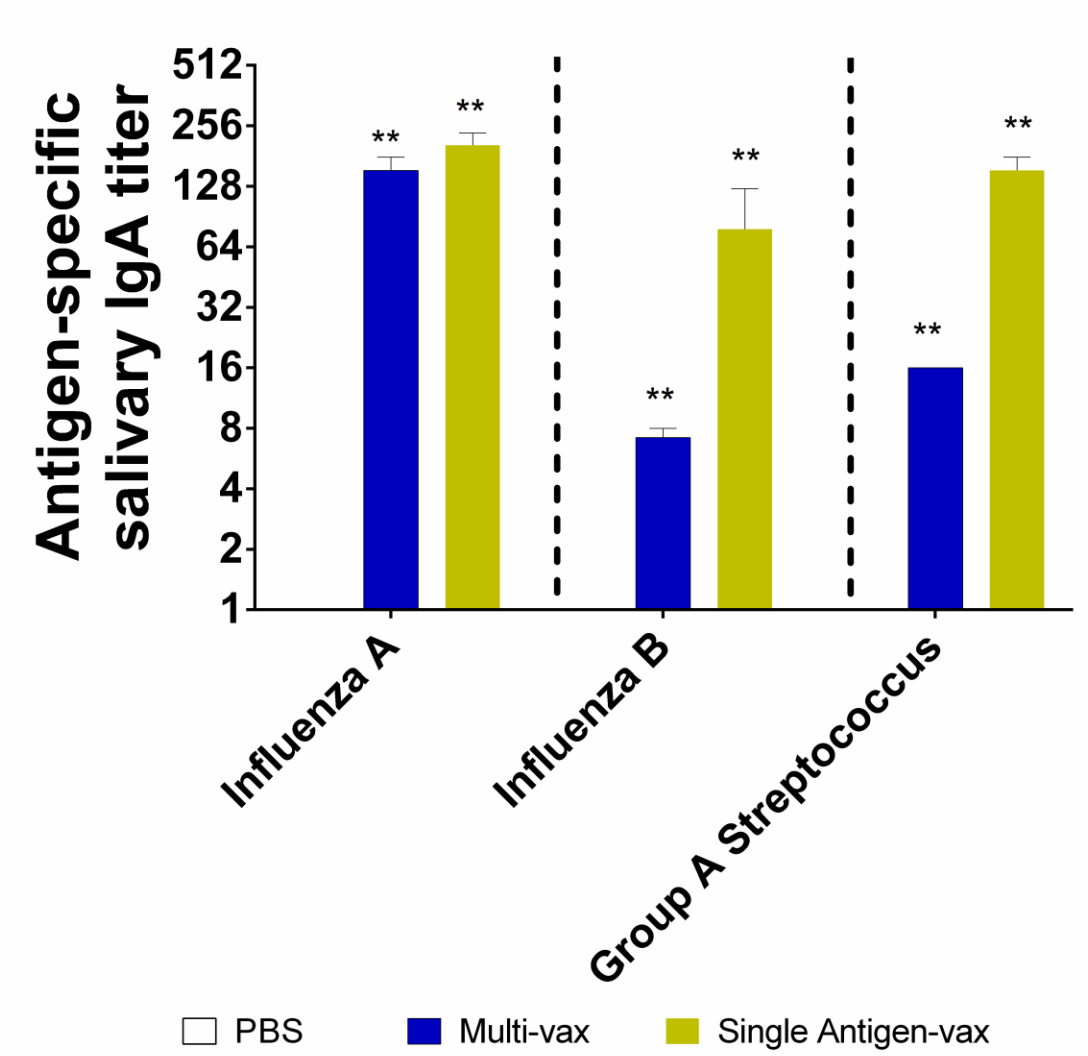
Influenza B (Bianchi E *et al*. J Virol. 2005;79(12):7380-8).

Group A Streptococcus (Batzloff MR *et al.* J Infect Dis. 2003;187(10):1598-608).



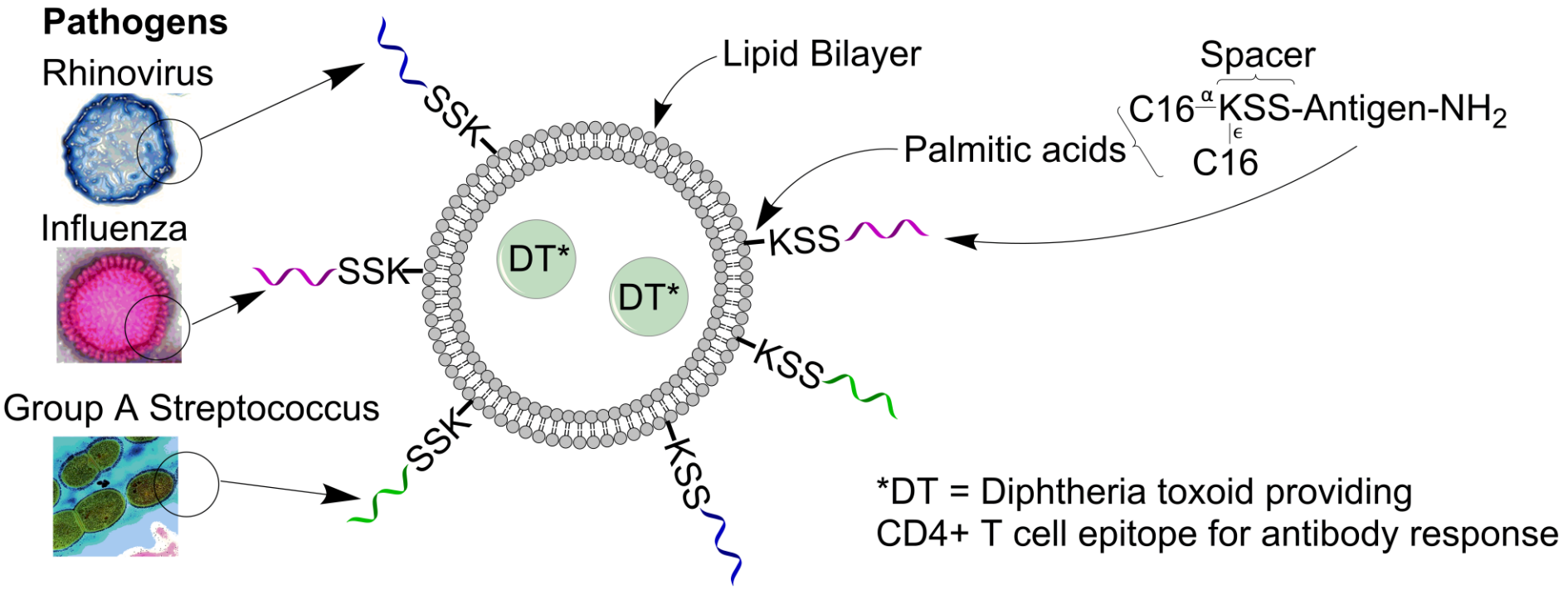
Multi-vax proof of concept: Immune response against multiple epitopes

- 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.



Acknowledgements

- Prof. Michael Good
- Dr. Michael Batzloff
- Dr. Manisha Pandey
- Victoria Ozberk
- Dr. Jennifer Reiman
- Emma Langshaw
- Prof. Geoff Hill
- Sai Lata De
- Virginia Mcphun, Ainslie Calcutt, Zac Phillips, Jessica Powell & Mei Fong Ho

- Research funding: