Lipophillic Nanoparticles as a Mechanism for Allergen Immunotherapy

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Abstract

The project objective was to develop working procedures to successfully synthesize adjuvant-conjugated lipophilic nanoparticles for allergen immunotherapy, as well as to better understand the mechanisms of allergen-antibody binding. To complete this task, the project was broken into three components. The first was to use Visual Molecular Dynamics and Nanoscale Molecular Dynamics to depict and simulate the mechanisms of the antibody-adjuvant complex. The second was to successfully develop lipophilic PEGylated nanoparticles, while the third was to determine the concentration of adjuvant necessary to inhibit the IgE antibody. Current results from this project include: the complex salt bridge amino acid composition, the area of the complex salt bridge and its impact on stability, replication in nanoparticle synthesis, and autofluorescence of nanoparticles under confocal microscopy. From this, the group determined that lipophilic nanoparticles were produced, and that targeting the salt bridge alters the stability of IgE.

Background

Problem:

- Allergic reactions are an immune system-mediated response to specific environmental allergen [2]
  - Response is mediated by Th2 helper cells, and has two phases:
  1. CD4+ Th2 cells produce IgE antibodies
  2. IgE binds to mast cells to produce allergic response when an allergen is recognized
- Over 15 million people suffer from allergic reactions in the United States alone [2]
  - Current treatments include allergy desensitization and *Omalizumab* therapeutic [1]

Project /Solution:

- Development of non-toxic and biodegradable IgE-inhibitory antibody-conjugated lipophilic nanoparticles to be inserted into the bloodstream to act as a preventative measure against allergic reactions

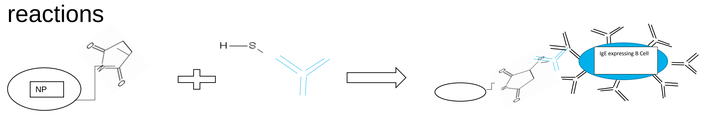


Figure 1: Model of anti-IgE binding to PEGylated nanoparticles, and then inhibiting IgE expressing B cells. Generated by PubMed Chemistry Drawing Tool. [3]

Project Objective

This project contains three main objectives:

1. Computational Simulation and Analysis of the complexes the IgE antibody is able to form and how it impacts therapeutic approach
   - Modeling of most stable conformation of complex
   - Comparison of IgE-allergen complex with project simulation (IgE-anti-IgE) and current allergen therapeutic (IgE-Omalizumab)
   - Determination of complex salt bridge area and amino acid composition
2. Technical development of lipophilic PEGylated nanoparticles
   - Successful replication of nanoparticles
3. Successful conjugation of goat Anti-IgE to lipophilic nanoparticles
   - Determination of concentration of adjuvant necessary to successfully inhibit IgE binding

Methods

Computational Analysis

Generic Buried Surface Area TCL script used to calculate salt bridge areas

Protein Databank Reference Files:

- 2r56 (Recombinant IgE Fab fragment complexed with Bovine Beta-Lactoglobulin Allergen)
- 5anm (IgE Fab complexed with neutralizing antibody [anti-IgE])
- 5hys (IgE complexed with Omalizumab

Nanoparticle Synthesis

- Protocol adapted from the work of Dr. Darrell J. Irvine of MIT
  - Anti-IgE is used as the adjuvant instead of Ovalbumin
  - Materials were used on a 10-fold scale to ensure the correct amounts of resources were used
  - Organic phase evaporated over a 19 hour period instead of a 12 hour period
- Particle synthesis [4]:
  - *poly lactic-co-glycolic acid (PLGA), 1,2-Dioleoyl-sn-glycero-3-phophocholine (DOPC), 1,2-Dioleoyl-sn-glycero-3-phosphoglycerol (DOPG),* and *1,2-distearoyl-sn-glycero-3-phophoethanolamine-N-[maleimide(polyethylene glycol)-2000] (mal-PEG2k-PE)* dissolved in *Dichloromethane (DCM)* to form organic phase
  - Deionized water dispersed into organic phase via sonification at 15 amps for 1 minute and 30 amps for 6 minutes
  - Solution incubated on stir plate for 19 hours at room temperature to evaporate DCM

Results

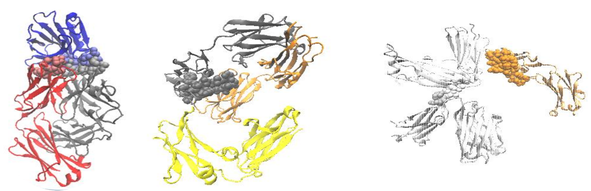


Figure 3: Visual Representation of IgE-Allergen Complex (Right), IgE-Inhibitor antibody Complex (Center) , and IgE-Omalizumab Complex (Left)

Salt Bridge Amino Acid Composition:

- IgE only contains amino acids with basic or neutral side chains
- 1 protein chain from each adjuvant contributes neutral amino acids
- Other adjuvant protein chain contains amino acids with acidic side chains
- Salt bridge forms via interactions between acidic and basic side chains

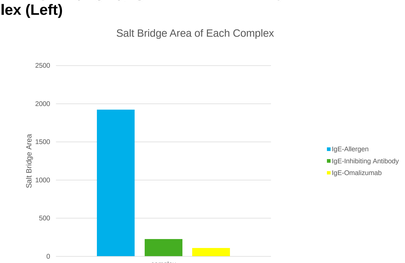
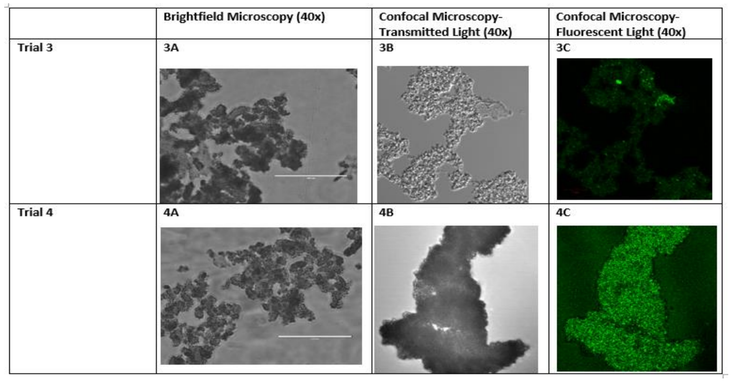


Figure 4: Area of the Amino Acid Salt Bridge for each IgE complex



	Brightfield Microscopy (40x)	Confocal Microscopy-Transmitted Light (40x)	Confocal Microscopy-Fluorescent Light (40x)
Trial 3	3A	3B	3C
Trial 4	4A	4B	4C

Figure 5: Microscopical analysis of Trial 3 and Trial 4 nanoparticle formation.
3A. 40x Brightfield Microscopy of Trial 3 nanoparticles; 3B. 40x Transmitted Confocal Microscopy of Trial 3 nanoparticles; 3C. 40x Fluorescent Confocal Microscopy of Trial 3 nanoparticles; 4A. 40x Brightfield Microscopy of Trial 4 nanoparticles; 4B. 40x Transmitted Confocal Microscopy of Trial 4 nanoparticles; 4C. 40x Fluorescent Confocal Microscopy of Trial 4 nanoparticles

Particles per µL calculation via ImageJ analysis

Assumptions:

1. Particle Diameter= 100µm
2. Use surface area of sphere for particle SA calculation ($SA= 4\pi r^2$)

Particle Surface Area: $SA= 4\pi(50nm)^2= 31415.93nm^2$

Nm to µm: $SA= \frac{31415.93nm^{\wedge}2}{1} X \frac{(0.001\mu m)^{\wedge}2}{1nm^{\wedge}2}= 0.0134\mu m^2$

To calculate number of particles present in image per µL:

$$\frac{Area\ of\ all\ particles\ (Image J)}{Surface\ Area\ of\ Particles\ (0.0134\mu m^2)^{*}20\mu L} X \frac{1}{} = \frac{nanoparticles}{\mu L}$$

- There were $\frac{8.76x10^4 particles}{\mu L}$ present in Trial 3
- There were $\frac{2.86x10^4 particles}{\mu L}$ present in Trial 4
- Trial 3 had about 4 times more nanoparticles per µL than Trial 4

Figure 6: Particles per µL calculation

Conclusion

Computational Analysis

- The area of the IgE-allergen salt bridge is about 10 times greater than the area of the IgE-inhibitory antibody complex, and 20 times greater than the IgE-Omalizumab complex
  - Salt bridges stabilize protein structures, so smaller salt bridges might lead to a more unstable and energetically-unfavorable complex [5]
  - Mast cell binding influenced by IgE stability [6]

Nanoparticle Synthesis

- As time from synthesis increases, nanoparticle aggregation also increases
- Trial 4 might have had fewer nanoparticles per µL due to less aggregation than Trial 3
- Both trials produced samples that auto fluoresced under confocal microscopy [7]

Future Directions

- Determine method to reduce aggregation of nanoparticles so older samples remain viable
- Conjugate adjuvant to nanoparticle to determine number of maleimide groups present on each nanoparticle
- Determine concentration of adjuvant necessary to inhibit IgE

Sources

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