Evaluation of Effector Functions of Antibodies Elicited by a Potential Broadly-Acting Influenza Vaccine

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BACKGROUND

Current Influenza Vaccines are Poorly Protective
- Annual deaths from influenza infection range from 12,000-52,000⁴
- Seasonal influenza vaccines have poor protection due to antigenic variation in the influenza virus
- Antigenic variation can occur from slow mutations, called antigenic drift, or reassortment between viruses, called antigenic shift.

Acetlated Dextran (Ace-DEX)
- Hydrophobic polymer that can form particles with potential for use as a vaccine delivery platform
- Tunable degradation (from hours to months)
- Degradation rate dependent on cyclic versus acyclic acetal coverage (CAC) (Fig. 1)²
- Acid sensitivity allows for the release of cargo at low pH and sustained release at neutral conditions

Figure 1. Ace-DEX (A) Acid-catalyzed synthesis of acetlated dextran from dextran: (B) Dependence of CAC on acetalization reaction time

Sustained-Release Ace-DEX CpG Adjuvant Offers the Best Protection Against Lethal Influenza Challenge in Mice
- CpG is an FDA-approved DNA oligonucleotide which binds to toll-like receptor 9 (TLR9) to cause immune cell activation and increase the effectiveness of vaccines
- CpG was formulated into Ace-DEX microparticles (MPs) to allow for control over its release profile and to target antigen-presenting cells, immune cells which preferentially take up larger particles and play a key role in activating the immune system.
- 20 CAC MPs exhibited immediate release of CpG while 60 CAC MPs exhibited sustained release over a month
- Vaccination of mice with fast or slow-releasing CpG MPs or soluble CpG mixed with influenza hemagglutinin (HA), resulted in similar HA binding antibody responses between fast and slow release CpGs
- Vaccination with slow release CpG MPs resulted in the greatest protection against lethal challenge (Fig. 1B)

Hypothesis: Differences in antibody effector function contribute to differences in protection.

METHODS

Antibody-Dependent Complement Deposition (ADCD)

Antibody-Dependent Cellular Phagocytosis (ADCP)

Figure 2. In vivo evaluation of different CpG Adjuvants with COBRA HA n=10 C57Bl/6 mice were vaccinated at days 0 and 21 with 1 µg hemagglutinin and 10 µg CpG (or Addavax) in the indicated formulations. (A) Serum Anti-HA antibody titers assessed at day 28 after vaccination. (B) Mice were challenged with 500,000 pfu Influenza A/California/07/2009

RESULTS

PBS
HA + Addavax
HA + CpG
HA + 20 CAC CpG MPs
HA + 60 CAC CpG MPs

Figure 3. Antibody-dependent phagocytosis and complement deposition (A) COBRA-conjugated beads were incubated with different dilutions of serum from mice vaccinated with indicated treatments, then used to treat J774A.1 macrophages for 4hr. Particle uptake by macrophages was quantified by flow cytometry (B) COBRA-conjugated beads were incubated with different dilutions of serum from mice vaccinated with indicated treatments, then incubated with guinea pig complement for 20 min. Fixed complement was detected with a FITC-conjugated anti-C3 antibody and the percentage of C3-positive beads was determined by flow cytometry.

Figure 4. Flow cytometry

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References

CONCLUSIONS

- Slow Release CpG resulted in the greatest amount of ADCP and ADCD.
- Future work will use these methods to investigate effector function of antibodies elicited by other adjuvant formulations.
- In the future, we will assess different effector functions such as ADCC (Antibody Dependent Cellular Cytotoxicity) and ADNP (Antibody Dependent Neutrophil Phagocytosis).
- Future studies will look at samples collected with a longer time after vaccination which we predict will lead to a greater development of effector function.