**Abstract:**
Vaccine adjuvants enhance the activity of weakly immunogenic recombinant proteins/antigens to achieve better potency, reduce the antigen load and also reduce the frequency of administration. Alum and emulsions, such as MF59 and AS03, are successful adjuvants for toxoids and recombinant antigens, respectively. In this study, we have examined three novel emulsion adjuvants similar in composition, but with different oil droplet size. The activity of these emulsions was compared to MF59 and key mechanistic features were identified.

**Introduction:**
Whole killed pathogens or live attenuated micro-organisms form the basis of vaccines. But with newer vaccine targets emerging, safer, well tolerated and easy to manufacture vaccines are needed. Subunit antigens although weakly immunogenic are a good alternative in comparison to conventional vaccines in terms of safety, tolerability and global need for immunization. Their weak immunogenicity is attributed to the fact that these subunit antigens have less number of Pathogen Associated Molecular Patterns (PAMPS) on their surface, and hence, cannot activate the immune system as much as a whole pathogen. Hence adjuvants are added to such weakly immunogenic antigens to help elicit a potent and effective immune response.

**Formulation and Characterization of Emulsion Adjuvants:**
Emulsions similar in components to MF59 were formulated, but with lower squalene oil content and different surfactant concentrations. The composition of MF59 is shown in Table 1. Three such emulsions were made, similar in concentration, but different in sizes. For simplicity purpose, we have named them as EA20, EA90 and EA160 (EA: emulsion adjuvants). The details of their sizes is presented in Table 2. Figure 1 presents a pictorial representation of these emulsion adjuvants (MF59, EA20, EA90 and EA160).

These novel emulsions were further characterized for pH, osmolality, endotoxin levels, number of large particles per mL and zeta potential. The details are presented in Table 3.

**Study 1:**
To understand the shape, size and structure of the novel emulsions we analyzed them using transmission electron microscopy (TEM). As previous measurement of size indicated the hydrodynamic diameter, we used TEM to size these emulsions. All emulsions were spherical in shape and had no unusual size distribution. Sizes obtained were comparable to the size measured by dynamic light scattering. The TEM images of these emulsions are presented in Figure 2.

**Study 2:**
6-8 weeks old female C57Bl/6 mice (total 21 with 3 animals/group) were immunized with 10µg OVA and adjuvants using the following groups:

- 10µg OVA + EA20
- 10µg OVA + EA90
- 10µg OVA + EA160
- 10µg OVA + Diluted MF59

**Key points of Study 1:**
- There was a boost in the titers after 2nd immunization.
- MF59 had the highest titer among all groups.
- All the novel adjuvants have activity close to diluted MF59 group.
- Immune responses among the novel adjuvants: EA 160 > EA 90 > EA 20

**Key points of Study 2:**
- Humoral responses were similar to Study 1 with EA 160 > EA 90, EA 20.
- Type of immune response analyzed by FACS (on spleenocytes) established that EA 160 has comparable quality of response to MF59 and diluted MF59 group.

**Conclusions:**
- MF59 had highest antibody titers in both animal studies and dilution of MF59 reduced the immunogenicity in mice for both Balb/c and C57Bl/6.
- Among the three novel adjuvants immunogenicity observed in terms of titers and quality of immune response.
- Although additional work is required to understand the relationship between size and type of immune response it can be concluded that adjuvant responses are dependent on concentration of oil and droplet size of emulsion adjuvants.

**References:**