

## Immune Adjuvant Activity of the Olive, Soybean and Corn Oils

Ana Claudia Marinho da Silva,<sup>1</sup> Erika Freitas-Mota,<sup>2\*</sup> José Lúcio Guerra,<sup>3</sup> Deijanira Albuquerque,<sup>4</sup> Maria Erivalda Aragão,<sup>5</sup> Diana Célia Sousa Nunes-Pinheiro,<sup>6</sup> Maria da Guia Silva-Lima,<sup>1</sup> Dirce Fernandes-Melo<sup>1</sup>

<sup>1</sup> Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará. CEP: 60455-900. Fortaleza, CE, Brasil.

<sup>2</sup> Departamento de Biologia, Universidade Federal do Ceará. Rua Humberto Monte, sn. Campus do Pici, Centro de Ciências, Bloco 906. CEP: 60455-900. Fax: +55 85 3366-9806. Fortaleza, CE, Brasil.

<sup>3</sup> Enza Zaden Beheer B.V. Haling 1E 1602 DB. Enkhueizen, The Netherlands.

<sup>4</sup> Departamento de Ciências Básicas em Saúde, Universidade Federal do Mato Grosso. CEP: 78000-900. Cuiabá, MT, Brasil.

<sup>5</sup> Laboratório de Biologia Celular, Bloco D. Universidade Estadual do Ceará. CEP: 60714-903. Fortaleza, CE, Brasil.

<sup>6</sup> Faculdade de Veterinária, Universidade Estadual do Ceará. CEP: 60740-002. Fortaleza, CE, Brasil.

**email:** erika.mota@ufc.br

---

In the last half of the century, a large amount of substances has been used as immune adjuvant. The immune adjuvant effect of olive, soybean and corn oils in Swiss mice immunized with ovalbumin (OVA) plus aluminum hydroxide or emulsified in Marcol, soybean, olive or corn oils was evaluated through the OVA-specific antibodies determined by ELISA and Passive Cutaneous Anaphylaxis. In this work the comparison of the intensity of the immune response was established by the Bayesian analysis. The adjuvant effect of the vegetable oils was shown to be more effective than aluminium hydroxide. Regarding to OVA-specific IgE synthesis, olive oil had the slowest adjuvant effect of the three vegetable oils. Accordingly, olive oil was the most convenient among the vegetable oils to be used as immune adjuvant, since it stimulated a higher production of OVA-specific Ig and lower levels of anti-OVA IgE.

**Keywords:** vegetable oils, adjuvants, antibody synthesis.

---

### Introduction

The development of recombinant protein and peptide immunogens has shown a major difficulty in preparing vaccines due to the weak immunogenicity of such antigens. Usually this obstacle has been overcome through the use of adjuvants whose study has been revealed as an attractive approach for enhancing immune responses, mainly when the immunogens are purified subunit antigens (1). Several substances have been tested as adjuvant to render vaccines more effective. For a considerable time, aluminium compounds were one of the few adjuvants licensed for use in human vaccines (2, 3). These adjuvants, however, neither stimulate a strong antibody response to all antigens nor stimulate cellular specific response, which is required to protect animals against virus and other intracellular infectious agents (2, 3). Thus, the study of new adjuvants still remains as an attractive field.

The use of mineral oil-based vaccines for animal has shown that they retained potency for a longer period of time than formulations containing aluminium

compounds (1-3). The enhanced effect of vaccines in water-oil emulsion is believed to derive from the gradual and continuous release of antigen to stimulate antibody production, the vehicle for transport of the antigen throughout the lymphatic system and the stimulus for the accumulation of immunologically important cells (4). Other aspect of adjuvant mechanism of action comprises: improving antigen presentation, inducing the production of immunomodulatory cytokines and increasing lifespan of activated T cells (1, 5). It is through the modulation exerted by cytokines that adjuvants promote the stimulation of the T-cell subsets that control the specific immune response: CD4<sup>+</sup> TH1 and TH2 and CD8<sup>+</sup> cells (6).

Concerning to the mechanism of action of the adjuvants it has also to be considered that it is attributed to them a role to preserve responding T cells what allows productive immune response to occur. Such effect is due, at least in part, to the increase of the expression of the protein Bcl-3 (nuclear factor NF- $\kappa$ B-I $\kappa$ B protein family) in T cells which may alter the balance of NF- $\kappa$ B transcriptional activities during immune response.

---

\* DSc, Professor, Universidade Federal do Ceará.

Thus Bcl-3 may play a role in the way the adjuvant, inflammation and innate immunity exert a control of the adaptive immune response (7).

Despite the important function played by adjuvant in the potentiation of the immune response, often-severe adverse reactions are reported in connection of its use. This is the case of fibrous tissue with small cyst-like spaces referred as granulomas, sterile abscesses, fistulous tracts, muscle atrophy, hypersensitivity pneumonitis, and embolic pneumonia (8), pyrogenicity, anterior uveitis, and arthritis (9).

Then, to select an appropriate adjuvant it is necessary not only avoiding unwanted side-effects but taking into consideration the requirements for humoral and cellular immune responses and also that adjuvant elicits both T-cell and B-cell memory.

The requirements for the choice of an appropriate adjuvant free of side effects is now easier since the knowledge of the cell types and cytokines interacting in immune response as well as the mode of action of adjuvant and the way they induce side-effects have been clarified.

Some vegetable oils have been used as immunological adjuvant (10, 11). The use of these oils has been criticized because there was some data showing they could cause contact allergy (12, 13).

However, this effect could be important since it triggers a TH1 response that is required to protect animals against virus and other intracellular infectious agents. Though, these oils are biologically degradable, their mentioned side effects have not been exhaustively investigated. Thus, they still remain as attractive candidates to be explored in the domain of adjuvant, a so prominent component in the induced immune response.

The aim of this work was to investigate the immune adjuvant effect of three vegetable oils, olive, soybean and corn in mice immunized with ovalbumin through the synthesis of specific antibodies.

The levels of OVA-specific IgE were also analyzed in view of possible side effects related to allergic reactions. The Nonconjugate Bayesian analysis of variance components (NBVC) was used to obtain maximum a posteriori estimators (MAE) (14).

## Materials and Methods

**Animals:** The 8-week-old female Swiss mice and adult rats used in this work were provided by Central Animal

House of the Federal University of Ceará, Brazil. The animals were kept in micro-isolators and all experiments have been conducted in accordance with the guideline for care and use of experimental animal of the National Counsel of Animal Experimentation Control (CONCEA-Brazil).

Antigens, plant oils and adjuvants: Ovalbumin, (OVA, grade V), was obtained from Sigma Chemical Co., St. Louis, USA. Marcol 52 (Exxon®) and commercial edible olive (Gallo®), soybean (Lisa®) and corn (Mazola®) oils were used after microbiological analysis and determination of fatty acid content.

### Determination of plant oils adjuvant activity on humoral immune response

Groups of 20 Swiss mice were immunized by subcutaneous injection with 10 mcg OVA (control) and with 10 mcg OVA with aluminum hydroxide (Al(OH)<sub>3</sub>) either emulsified to marcol, olive, soybean or corn oils.

A negative control was followed by saline injection. The mice were bled from the orbital plexus on days 7, 14, 21, 28, 35, 42 after the first injection.

Booster injections were done on 21st and 35th day in the same conditions as in primary immunization. The sera were individually collected and stored at -20°C until usage. The OVA-specific antibody levels (IgG, IgA and IgM) were assayed by ELISA using sera from each animal of different groups, as previously described by Sartor et al. (15).

The OVA-specific IgE was estimated in rats by Passive Cutaneous Anaphylaxis (PCA) (16).

The interval between skin sensitization and challenge was 18 h. For challenge, an intravenous injection of 1 mL of a solution containing 0.5% Evans blue in saline and 2.0 mg OVA was used. PCA titers were expressed as log 2 of the inverse of the highest dilution giving a positive reaction. Each serum sample was assayed in at least 2 rats.

Controls were performed in every rat with a known standard serum: the reaction given by the sera tested was weighted in relation to the titer of the standard serum (17). Controls without serum or antigen in the challenging solutions gave negative results.

### Statistical analysis

The results were treated by the nonconjugate Bayesian analysis of variance components (NBVC) that was used to obtain maximum a posteriori estimators (MAE) (14).

The full statistical model is:

$$y = Xb + Zu + \text{error (Equation 1)}$$

Z is a designed matrix; u will model the non-linear effects of time (7, 14, 21, 28, 35 and 42 days) as random effects; X is a designed matrix; b will model the fixed effects of treatments; error is our estimate of experimental error.

## Results

Table 1 consists of the descriptive statistics of the data obtained from ELISA titers of serum levels of antibodies and from PCA titers of IgE in mice immunized with OVA in presence or not of different types of adjuvant in a balance designed with the experimental units observed over time. Animals injected with saline did not exhibit OVA-specific antibodies.

Marcol stimulated a higher production of anti-OVA antibodies, followed by the groups immunized with OVA associated with Al(OH)<sub>3</sub>, or emulsified in olive, soy and corn oils. There was no difference on the adjuvant effect among the three oils (Table 1).

Regarding the anti-OVA IgE levels, Al(OH)<sub>3</sub> stimulated a higher production of anti-OVA IgE, followed by Marcol. Among the oils, the soybean one stimulated the highest production of anti-OVA IgE when compared to corn and olive oils.

The serum levels of antibodies induced by ovalbumin in the presence of the several adjuvants e.g. marcol, Al(OH)<sub>3</sub> and vegetable oils compared to OVA injected alone is shown in Table 2. When comparing each group reported to total anti-OVA, different behaviors were revealed.

For contrast 1 (Corn oil vs Olive oil) it was observed a MAE of 0.001, which does not show any significant difference between the adjuvant effects of the oils. A MAE of 1.61 (p<0.05) for contrast 2 (Al(OH)<sub>3</sub> vs Control), 3.24 (p<0.05) for contrast 3 (Marcol vs Control), 2.23 (p<0.05) for contrast 4 (Soybean Oil vs Control), 1.63 (p<0.05) for contrast 5 (Al(OH)<sub>3</sub> vs Marcol) and 2.63 (p<0.05) for both, contrast 6 (Corn Oil vs Control) and 7 (Olive oil vs Control) were also obtained. The specific IgE synthesis induced by ovalbumin in the presence of the several adjuvants e.g. marcol, Al(OH)<sub>3</sub> and vegetable oils compared to injected OVA without adjuvant is shown in Table 3.

A MAE of 2.2 (p<0.05) for contrast 1 (Corn oil vs Olive oil), 3.52 (p<0.05) for contrast 2 (Al(OH)<sub>3</sub> vs Control), 3.41 (p<0.05) for contrast 3 (Marcol vs Control), 2.70 (p<0.05) for contrast 4 (Soybean oil vs Control), 0.10 (p<0.05) for contrast 5 (Al(OH)<sub>3</sub> vs Marcol), 2.20 (p<0.05) for contrast 6 (Corn oil vs Control), 1.52 (p<0.05) for contrast 7 (Olive oil vs Control) were obtained.

**Table 1.** OVA-specific Ig (IgG, IgA and IgM) evaluated by ELISA and IgE determined by PCA in mice immunized with ovalbumin in the presence of different adjuvants: descriptive statistics.

OVA-specific Ig	Adjuvant	Mean Values	Standard deviation	N <sup>1</sup>
<b>Ig (IgG, IgA, IgM)</b>	Control	9.88	5.73	120
	Al(OH) <sub>3</sub>	11.50	5.97	120
	Marcol	13.13	6.60	120
	Corn oil	12.12	5.73	120
	Olive oil	12.12	6.45	120
	Soybean oil	12.12	6.09	120
<b>IgE</b>	Control	3.58	2.68	120
	Al(OH) <sub>3</sub>	7.11	2.09	120
	Marcol	7.05	1.92	120
	Corn oil	5.79	3.66	120
	Olive oil	5.11	2.79	120
	Soybean oil	6.28	3.64	120

Mice were immunized by subcutaneous route with OVA alone or associated to Al(OH)<sub>3</sub>, Marcol, or vegetable oils. The results were expressed as mean values of different treatments (Marcol, Al(OH)<sub>3</sub>, olive, corn and soybean oils) throughout 7, 14, 21, 28, 35, 42 days and treated by NBVC as previously described.

<sup>1</sup>There are 20 individuals assigned to 6 treatments.

**Table 2.** Selected contrasts for OVA-Ig specific levels.

Contrasts	MAE <sup>1</sup>	95% CCI <sup>3</sup> for LSD <sup>2</sup>
(1) Corn oil vs. Olive oil	0.001	0.51 to -1.26
(2) Al(OH) <sub>3</sub> vs. Control	1.61	2.16 to 0.48*
(3) Marcol vs. Control	3.24	3.76 to 2.06*
(4) Soybean oil vs. Control	2.23	2.76 to 1.08*
(5) Al(OH) <sub>3</sub> vs. Marcol	1.63	-0.92 to -2.87
(6) Corn oil vs. Control	2.63	2.96 to 0.94*
(7) Olive oil vs. Control	2.63	2.76 to 1.49*

<sup>1</sup>MAE: Maximum Posteriori Estimators<sup>2</sup>LSD Least Square Difference<sup>3</sup>CCI central credibility interval

\*p&lt;0.05

The analyzed data were obtained from ELISA.

## Discussion

In this work we experimentally demonstrate the adjuvant effect of three vegetable oils: olive, soybean and corn oils. The presence of fungi and bacteria was not revealed by the microbiological analysis of corn, soybean and olive oils. Concerning the composition of saturated and unsaturated fatty acids the three vegetable oils present different profiles. The contents of oleic acid are 85, 50 and 29% in olive, corn and soybean oils, respectively and the contents of linoleic acid are 5, 34 and 57% in olive, corn and soybean oils, respectively as it has been already demonstrated by Moreto (18).

The initial statistical demonstrate that Marcol had the largest adjuvant effect on the synthesis of antibodies, including IgE (Table 1). This result was confirmed by the mathematical model (Equation 1) that adjusted the raw data for the source of variation associated with the time (Tables 2 and 3).

Hence, there is strong evidence supporting the idea that Marcol had the largest effect on the synthesis of antibodies. Negative effects were not observed as they were compared to the control. It was also shown that there is not much difference among them when the averages are compared. Concerning to IgE synthesis, contrast 7 (Olive oil vs Control) shows that olive has the lowest possible values for the MAE when compared to control (Table 3). Also olive oil stimulated OVA-specific IgE synthesis significantly smaller than corn oil for example. The adjuvant effect of Al(OH)<sub>3</sub> for the IgE response has clearly the largest value as it is already known (19). Also from contrast 5 (Al(OH)<sub>3</sub> vs Marcol), we can see that the

**Table 3.** Selected contrasts for OVA-specific IgE synthesis.

Contrasts	MAE <sup>1</sup>	95% CCI <sup>3</sup> for LSD <sup>2</sup>
(1) Corn oil vs. Olive oil	2.2	2.44 to -1.64
(2) Al(OH) <sub>3</sub> vs. Control	3.52	3.82 to 2.81*
(3) Marcol vs. Control	3.41	3.66 to 2.70*
(4) Soybean oil vs. Control	2.70	2.94 to 2.16*
(5) Al(OH) <sub>3</sub> vs. Marcol	0.10	0.40 to -0.59
(6) Corn oil vs. Control	2.20	2.44 to 1.64*
(7) Olive oil vs. Control	1.52	1.79 to 0.92*

<sup>1</sup>MAE: Maximum Posteriori Estimators<sup>2</sup>LSD Least Square Difference<sup>3</sup>CCI central credibility interval

\*p&lt;0.05

The analyzed data were obtained from PCA.

difference between Al(OH)<sub>3</sub> and Marcol is not significant (p<0.05) (Table 3).

These results put in evidence the potentiality of the vegetable oils as adjuvants in experimental protocols of immunization/vaccination whose antigens are supposed to require TH2-dependent immune response, as it is the case of ovalbumin. The adjuvant effect of the vegetable oils was consistently larger than the effect of aluminum, one of the five well established vaccine adjuvants (2, 20). In addition, olive oil stimulated smaller amount of OVA-specific IgE comparing to Al(OH)<sub>3</sub>, which is known as an adjuvant that stimulates large levels of IgE against OVA (19). Moreover, rice oil has proven their adjuvant activity previously (15). Although the local inflammation and formation of small granulomas are considered to be essential for the adjuvant effect (17), this inflammatory reaction is frequently referred as an inconvenient in the vaccination procedures. Indeed, vegetable oils used did not induce macroscopically granulomatous response as was proven by the daily observation of the injection site.

## Conclusion

The potentialities of the vegetable oils as immunological adjuvants in vaccination procedures are increasing since they are biologically degradable. This advantage is added to their strong adjuvant capacity as it has been demonstrated in the present work revealing that olive oil is the most convenient among the vegetable oils evaluated in this work to use as immune adjuvant. Such conclusion is supported by the results showing a strong OVA-specific Ig and a weak OVA-specific IgE stimulated by olive oil.

## Acknowledgements

This work was supported by grants from CAPES, FUNCAP and CNPq.

## References

1. Vogel FR. Adjuvants in perspective. *Developments in Biological Standardization* 1998;92:241-8.
2. Engers H, Kieny MP, Malhotra P, Pink JR. Third meeting on Novel Adjuvants Currently in or Close to Clinical Testing World Health Organization – Organisation Mondiale de la Santé, Fondation Merieux, Annecy, France. *Vaccine* 2003;21:3503-24.
3. Brito LA, Malyala P, O'Hagan DT. Vaccine adjuvant formulations: A pharmaceutical perspective. *Seminars in Immunology* 2013;25:130-45.
4. Barnett PV, Pullen L, Williams L, Doel TR. International bank for Foot-and-mouth disease vaccine: assessment of Montanide ISA 25 and ISA 206, two commercially available adjuvants. *Vaccine* 1996;14:1187-98.
5. Cox JC, Coulter AR. Adjuvants – a classification and review of their modes of action. *Vaccine* 1997;15:248-56.
6. Audibert FM, Lise LD. Adjuvants: current status, clinical perspectives and future prospects. *Immunology Today* 1993;14:281-4.
7. Mitchell TC, Hildeman D, Kedl RM, Teague TK, Schaefer BC, White J, et al. Immunological adjuvants promote activated T cell survival via induction of Bcl-3. *Nature Immunology* 2002;2:397-402.
8. Claassen E, Leeuw W, Greeve P, Hendriksen C, Boersma W. Freund's complete adjuvant: an effective but disagreeable formula. *Research in Immunology* 1992;143:478-83.
9. Allison AC, Byars NE. Immunological Adjuvants: desirable properties and side effects. *Molecular Immunology* 1991;28:279-84.
10. Brugh M, Stone HD, Lupton HW. Comparison of inactivated Newcastle disease viral vaccines containing different emulsion adjuvants. *American Journal of Veterinary Research* 1983;44:72-5.
11. Kimura J, Nariuchi H, Watanabe T, Matuhasi T, Okayasu I, Hatakeyama S. Studies on the adjuvant effect of water-in-oil-in-water (w/o/w) emulsion of sesame oil. 1. Enhanced and persistent antibody formation by antigen incorporated into the water-in-oil-in-water emulsion. *The Japanese Journal of Experimental Medicine* 1978;48:149-54.
12. Basketter D, Kimber I. Olive oil: suitability for use as a vehicle in the local lymph node assay. *Contact Dermatitis* 1996;35:190-1.
13. Crevel RWR, Kerckhoff MA, Koning MM. Allergenicity of refined vegetable oils. *Food and Chemical Toxicology* 2000;38:385-93.
14. Wolfinger RD, Kass RE. Nonconjugated Bayesian analysis of variance components models. *Biometrics* 2000;56:268-77.
15. Sartor ITM, Colodel EM, Albuquerque D. Adjuvant activity of rice oil on the immune response to ovalbumin. *VacchiMonitor* 2011;20(2):1-5.
16. Mota I, Wong D. Homologous and Heterologous Passive Cutaneous Anaphylactic Activity of Mouse anti-sera During the Course of Immunization. *Life Science* 1969;8:813-20.
17. Prouvost-Danon A, Mouton D, Abadie A, Biozzi G. Genetic regulation of IgE and agglutinating antibody synthesis in lines of mice selected for high and low immune responsiveness. *European Journal of Immunology* 1977;7:342-8.
18. Moreto, E. *Tecnologia de óleos e gorduras*. São Paulo: Ed. Varela; 1998.
19. Prouvost-Danon A, Binaghi R, Rochas S, Boussac-Aron Y. Immunochemical identification of mouse IgE. *Immunology* 1972;23:481-91.
20. Brewer JM, Conacher M, Gaffney M, Douglas M, Bluethmann H, Alexander J. Neither interleukin-6 nor signaling via tumor necrosis factor receptor-1 contribute to the adjuvant activity of Alum and Freund's adjuvant. *Immunology* 1998;93:41-8.

---

## Actividad inmunoadyuvante de los aceites de oliva, soja y maíz

### Resumen

En la última mitad del siglo; una gran cantidad de sustancias han sido utilizadas como inmunoadyuvantes. El efecto inmunoadyuvante de los aceites de oliva, de soja y maíz en ratones suizos inmunizados con ovoalbúmina (OVA), además de hidróxido de aluminio o emulsionados en Marcol se evaluó a través del método de Elisa utilizando anticuerpos-OVA específicos y mediante la prueba de anafilaxis pasiva cutánea. En el presente trabajo, se estableció la intensidad de la respuesta inmune de los distintos tratamientos mediante análisis Bayesiano. El efecto adyuvante de los aceites vegetales ha demostrado ser más eficaz que el hidróxido de aluminio. El aceite de oliva tuvo el efecto adyuvante más lento de los tres aceites vegetales en cuanto a la síntesis de IgE OVA-específicos. Por consiguiente, el aceite de oliva fue el más conveniente entre los aceites vegetales para ser utilizado como inmunoadyuvante, ya que estimuló una mayor producción de IgG OVA-específicos y niveles más bajos de IgE anti-OVA.

**Palabras claves:** aceites vegetales, adyuvantes, producción de anticuerpos.

---