

# Nano-structured Vaccine Delivery

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## ABSTRACT

The nano-structured vaccine delivery system, containing tomatine, n-octyl- $\beta$ -D-glucopyranoside (OGP), phosphatidylethanolamine (PE), cholesterol, and ovalbumin (OVA), was examined in the present study. Transmission electron microscopy (TEM) and fluorescence microscopy were carried out to examine the nano-structures of this complex vaccine delivery system and its interactions with the antigen-presenting cells (APCs).

The adjuvant was prepared and separated by isopycnic ultracentrifugation. Examination by transmission electron microscopy revealed the heterogeneity characteristics, containing several micro- and nano- structures with the major fraction containing needle- and rod-shaped nanoparticles of approximately 80-160 nm in width and 2-4  $\mu$ m in length. Tomatine alone in 0.9% NaCl, on the other hand, was shown to be cylindrical crystals of hundreds of nanometers up to a few micrometers in length. The nano-gel structures were speculated to correlate with the immunostimulating effect of the adjuvant.

To examine the roles of the adjuvant in antigen delivery, dendritic cells (DCs) were isolated from bone marrows of mice and co-cultured with tomatine adjuvant containing the fluorochrome-labeled antigens (Ags). Confocal fluorescence microscopic examination demonstrated the uptake of tomatine-antigen complexes by the DCs, suggesting the roles of the nano-structured vaccine adjuvant in antigen delivery.

**Keywords:** adjuvant, vaccine, antigen delivery

## 1. INTRODUCTION

The vaccine adjuvant, containing tomatine, n-octyl- $\beta$ -D-glucopyranoside (OGP), phosphatidylethanolamine (PE), cholesterol, and ovalbumin (OVA), has been shown to elicit cytotoxic T-lymphocyte (CTL) effect when co-immunized with the antigens (Ags). To elucidate the microstructures, this adjuvant was separated by isopycnic ultracentrifugation, followed by examination by transmission electron microscopy (TEM). The adjuvant was shown to be mixtures of several micro- and nano- structures, serving as the micro-templates for complexation with the antigens. Several other forms of micro- and nano-structures were observed, showing multiple dispersion features with the nano-gel characteristics.

To elucidate the interactions between the adjuvant and the antigen presenting cells (APCs), dendritic cells (DCs), the most powerful antigen presenting cells, were isolated from mice and incubated with the tomatine adjuvant containing the fluorochrome-labeled antigens, and examined by a confocal fluorescence microscope.

## 2. EXPERIMENTAL

The (tomatine/ovalbumin) adjuvant-antigen complex was prepared as described in the previous studies. Briefly, Mixture A containing tomatine, n-octyl- $\beta$ -D-glucopyranoside (OGP), phosphatidylethanolamine (PE) in 0.9% NaCl was vortexed and heated to 60 °C until all the components were dissolved. Mixture B containing cholesterol, OGP, and PE in 0.9% NaCl was heated to 60 °C until all the components were dissolved and then incubated at 37 °C in a water bath. Mixture C was prepared by

dissolving ovalbumin in 0.9 % NaCl. Mixture C was mixed with Mixture A and incubated at 37 °C, Mixture B was then added to this mixture and vortexed vigorously for 10 minutes. The final tomatine adjuvant mixture was then dialyzed against 0.9% NaCl for 24 h. To examine the nano-structures of this vaccine adjuvant, the adjuvant mixture was separated by isopycnic ultracentrifugation and examination by transmission electron microscopy.

To study the delivery functions of the nano-structured vaccine adjuvant in the antigen presenting cells (APCs), ovalbumin (OVA) was dissolved in 0.1 M sodium carbonate (pH 9.0) and labeled with fluorescein isothiocyanate (FITC), followed by gel filtration through a Sephadex G-50 column. Bone marrow derived dendritic cells (DCs) were isolated from the male C57BL/6J mice at 6-8 week of age according to the established procedures. DCs were cultured for 6 days in RPMI containing  $\beta$ -mercaptoethanol, HEPES, fetal bovine serum, antibiotics, and supplemented with mGM-CSF and mIL-4. The DC phenotype was confirmed by staining with the antibodies and subsequently analyzed by flow cytometry. To facilitate microscopic observations, the dendritic cells were labeled with rhodamine-phalloidin and incubated with tomatine adjuvant containing the fluorochrome-labeled antigens, followed by examination by a Zeiss Axiophot 2 fluorescence microscope.

### 3. RESULTS

#### **TEM examination of fractions of tomatine adjuvant by isopycnic ultracentrifugation**

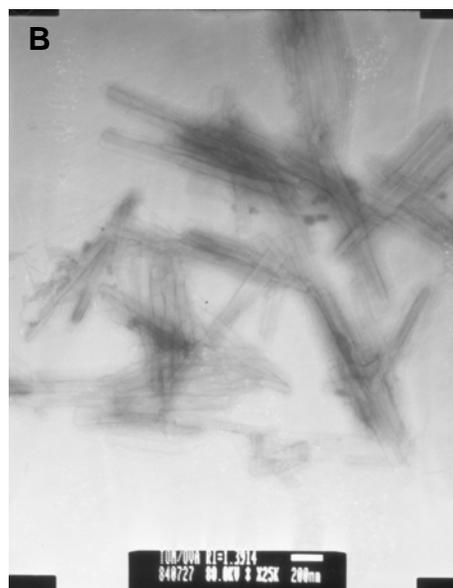
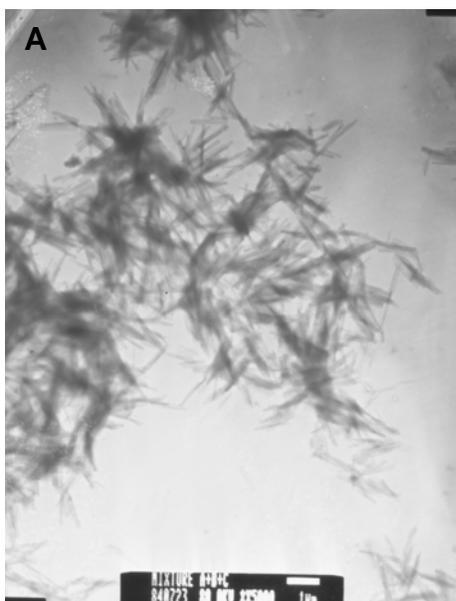
The tomatine adjuvant was subjected to isopycnic ultracentrifugation. At least ten fractions were extracted from each centrifuge tube after ultracentrifugation. Examination by transmission electron microscopy revealed the heterogeneous micro- and nano- structures. The major band observed at refractive index (RI) = 1.398 in the sucrose gradient exhibited aggregated needle-shaped particles, ranging from 2 to 4  $\mu$ m in

length and approximately 80-160 nm in width (Fig 1A). In the fraction at RI = 1.3914, transparent rod-shaped crystalline particles were observed (Fig. 1B). The average size of these cylindrical complexes was larger than the individual tomatine particles in 0.9% NaCl aqueous dispersion (Fig. 2).

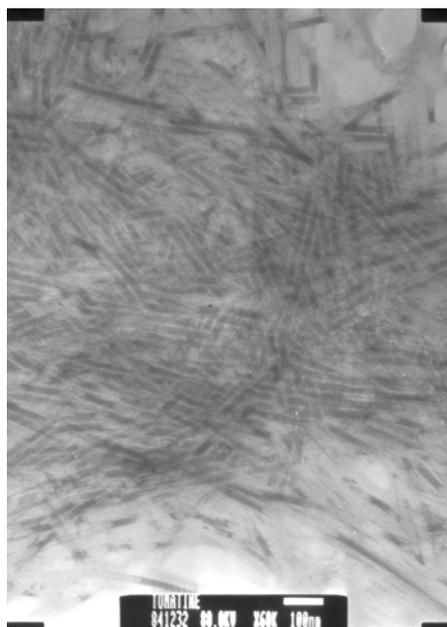
The fraction below the major band at RI = 1.418 shows the polydispersed nano-gel structures (Fig. 3). In the fractions above the major band at RI = 1.3839, aggregated and polydispersed nano-gel structures of a wide size range, varying from a few nanometers up to hundreds of nanometers, were observed (Fig. 4A). In the fraction at RI = 1.3728, several nanospherical vesicles were distributed throughout the gel matrix (Fig. 4B). The morphological characteristics shown in these figures illustrated the polydispersed nano-gel structures and the multiple-dispersion characteristics, suggesting the roles of the vaccine adjuvant in delivering the antigens.

#### **Phagocytosis of tomatine-antigen complexes by the DCs**

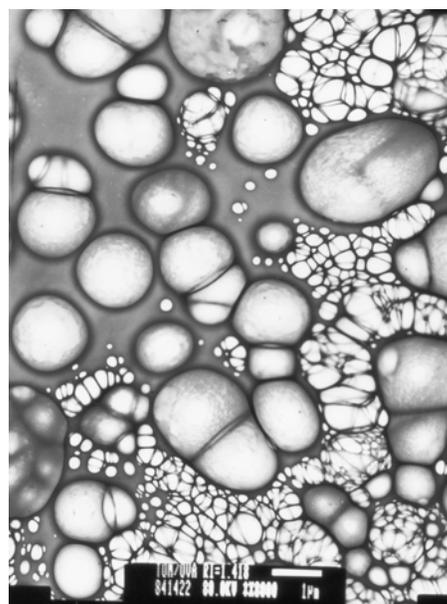
To examine the uptake of the Ag-tomatine complexes by the antigen presenting cells, dendritic cells (DCs) isolated from the femurs and tibias of male C57BL/6J mice were cultured for 6 days, and labeled with rhodamine-phalloidin, followed by incubation with the tomatine adjuvant containing the FITC-labeled antigens. Examination by confocal fluorescence microscopy showed that DCs phagocytosed the antigen containing adjuvant. Our previous studies showed that tomatine adjuvant induces both apoptosis and necrosis in EL4 thymoma cells. Results obtained in this study demonstrated that tomatine adjuvant not only induces cell death but also delivers the antigens into the antigen presenting cells, suggesting the roles of the nano-structured vaccine adjuvant in antigen delivery.



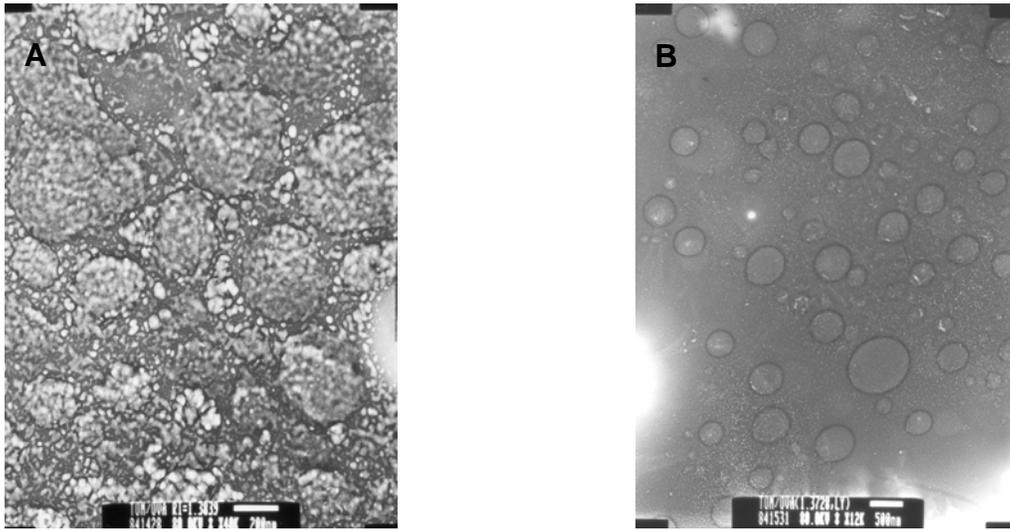
**Fig. 1** Transmission electron micrographs of the micro- and nano- structures in the major bands after isopycnic ultracentrifugation, showing the aggregated needle- and rod-shaped nanostructures of the tomatine adjuvant.



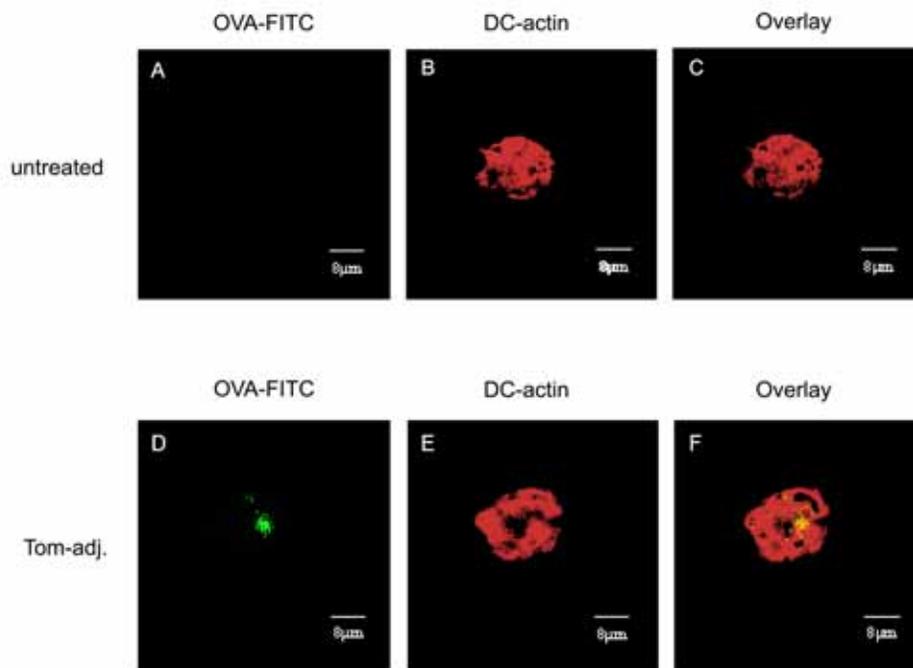
**Fig. 2** Transmission electron micrograph of tomatine in 0.9% NaCl, showing the cylindrical nano-structures.



**Fig. 3** Transmission electron micrographs of the fractions at RI = 1.418, showing the nano-gel structures of the tomatine adjuvant



**Fig. 4** Transmission electron micrographs showing the presence of (A), the nano-gel structures (RI = 1.3839) and (B), the dispersed matrix containing the nano-spherical vesicles (RI = 1.3728), in the tomatine adjuvant.



**Fig. 5** Phagocytosis of tomatine adjuvant, containing FITC- OVA, by the dendritic cells.

## REFERENCES

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