

Protocol for Macrophage Depletion from Mice

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[Abstract] Macrophage depletion has been used extensively to study autoimmune disease and more recently in tumor models. The clodronate-containing liposomes will be recognized as foreign particles and get engulfed by macrophages upon dosing into the animal by the chosen routes. Consequently, macrophages that have engulfed liposomes will all be destroyed by the liposomal. In the protocol presented here the clodronate-containing liposomes were used to systemically deplete macrophages in mice.

Materials and Reagents

1. Mice
2. Clodronate (dichloromethylene diphosphonic acid)-loaded liposomes (VUmc)
3. PBS-containing liposomes (VUmc)
4. Phosphatidyl choline (Lipoid GmbH, catalog number: D-67065)
5. Cholesterol (Sigma-Aldrich, catalog number: C8667)
6. Liposomes (see Recipes)
7. Phosphate buffered saline (see Recipes)

Equipment

1. Syringe (10 ml and 1 ml) (BD, catalog numbers: 309604 and 305111)
2. 26 G 1/2 needles (BD, catalog number: 309659)

Procedure

1. Twenty, 8-week-old mice were randomly divided into two groups.
2. One group of mice were intravenously injected using 1-ml syringe with 26 G 1/2 needles, twice per week with clodronate-loaded liposomes in 150 μ l for the first month and 100 μ l following the first month's injection to reduce possible side effects, such as infection, live toxicity *et al.* The mice were treated for 3 months totally.
3. The second group was treated with PBS-liposomes.

4. At different time points (such as 2, 4, 8 weeks after treatments) of the experiment, one or two mice from each group will be euthanized to examine inflammation and other interesting analysis. Based on our experiments, the FACS analysis detected around 90% of macrophage reduction in the group that treated with the clodronate-loaded liposomes versus the control group after 2 weeks treatment, and this reduction kept to the end of the experiments.
5. During the whole treatment period, the treated mice need to be closely monitored for the possible signs and symptoms such as weight loss, yellow, bloody, or tar-colored stool, dark urine color, diarrhea, fatigue etc.
6. At the end of the experiment, (depend on the experiment), the mice were euthanized and the lung tissues were collected for various analyses.

Recipes

Notes:

- a. *Based on the instructions from company.*
 - b. *The clodronate-loaded liposomes and PBS-containing liposomes are ready for use, but the solutions that usually kept in 4 °C need to be warmed up to room temperature before injections.*
1. Liposomes

Liposomes are artificial lipid spherules made of:

 - a. Phosphatidyl choline
 - b. Cholesterol
 2. Phosphate buffered saline (PBS)
 - a. 10 mM Na₂HPO₄
 - b. 10 mM NaH₂PO₄
 - c. 140 mM NaCl

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