# PREPARATION OF ANTI-LEUKEMIC T-CELL IMMUNOLIPOSOMES AND THEIR TARGET SPECIFIC PROPERTIES

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Leukemic cells exist in blood, they are easy to get in touch with target sensitive drugs. Liposomes have been extensively tested as effective, non-toxic drug carriers. Immunoliposomes, which consist of liposomes and highly specific antibodies, have been shown to bind specifically to their target cells in vitro and in vivo.

In the work presented in this note, we have combined an anti-human T leukemic cell immunoliposome with cytotoxin from Chinese cobra (CT) as a "target-directed" pharmaceutical. Thermotropic phase transition of the immunoliposome was determined by differential scanning calorimetry. The cytotoxic properties of the immunoliposomes on leukemic cells CEM were evaluated.

## I. MATERIALS AND METHODS

### 1. *Materials*

Antibody Wu71 against whole human T lymphocytes was isolated from mouse ascites fluid and kindly provided by Dr. Shi[1]. Cytotoxin from Chinese cobra (CT) was isolated and kindly provided by Dr. He[2]. Dioleoylphosphatidylethanolamine (DOPE) and Oleic acid (OA) were purchased from Sigma Chemical Co. N-hydroxysuccinimide ester of stearic acid (NHSS) was obtained from Department of Chemistry, Beijing University. The human T leukemic cell line CEM was used as target, and the human B lymphocyte Raji served as the non-target control to evaluate the selectivity of immunoliposome-mediated cytotoxicity. CEM expressed Wu71 antigen, while Raji did not. Calcein was obtained from Serva Chemical Co.

### 2. *Methods*

1) Derivatization of antibody. Coupling of the NHSS to antibody was done following the procedure of Huang et al.[3], i.e. 2-mg antibody Wu71 was added to 44-$\mu$g NHSS in phosphatebuffered saline (PBS, pH7.5) such that the final deoxycholate concentration was 0.25%.

The coupling was performed at 37°C for 16 h and dialyzed against PBS (pH 7.5) for 48 h.

2) Immunoliposome preparation[4]. Solvent-free lipid films containing DOPE/OA (8:2) were suspended in PBS containing 50 mmol/L calcein or/and 0.4 mol/L CT. The lipid suspension was sonicated at room tempareture for 10 min with a bath sonicator (Laboratory Supplies Co. Inc., G112 SPIT) and the pH was adjusted to 7.6.

Derivatized IgG was then added to the sonicated lipid at a lipid to protein ratio of 10:1 (w/w). After further sonicating, the mixture was dialyzed, the liposome suspension was then chromatographed on a column of Bio-gel A 0.5 mol/L (1.1×30 cm) to remove unincorporated calcein, CT and IgG.

3) Test pH-sensitivity of immunoliposomes by fluorescence measure. 20 μl immunoliposomes containing calcein were suspended in 2-ml PBS whose pH were 8.0, 7.2, 6.0, 5.0 and 3.5 respectively. The fluorescence intensity of immunoliposomes was measured (wavelength 490 nm) using a Hitach fluorometer.

4) Thermotropic phase transition of the immunoliposomes assay[5]. 40-μl immunoliposomes containing derivatizated IgG (one containing CT and another not containing CT) were suspended in PBS. The thermotropic phase trasition curve (from −4°C to 40°C) was established by differential scanning calorimetry[5] using a Du Pont 1090 B thermal imaging system.

5) Incubation of immunoliposomes with cells. CEM and Raji cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Liposomes carrying either calcein or CT were incubated with $1\times10^5$ /ml CEM or Raji cells at 37°C for 2 h. The cells were washed twice with the medium, then incubated in fresh medium for an additional 8 h at 37°C.

The fluorescence intensity was observed under the fluorescence microscope. The cell mortality was determined by using acridine orange/ethidium bromide (AO/EB) staining.

In some experiments, cell-liposome incubation was done at 4°C for 2 h when the unbound liposomes were washed away twice with the medium. The cells were then incubated in fresh medium at 37°C as above.

6) Pretreatment of cells with free antibody. Free antibody Wu71 at a concentration of $10^{-9}$ mol/L was incubated with CEM cells at 37°C for 30 min. The cells were washed once and then incubated in fresh medium at 37°C before adding immunoliposomes and observed in the same way as above.

## II. Results and Discussion

### 1. *Stability of the Immunoliposomes*

The average size of the immunoliposomes composed of DOPE/OA was 27.7±11.3 nm determined by electron microscopy (Fig. 1). Fig. 2 shows that the immunoliposomes are

Fig. 1. Immunoliposomes under scanning electron microscope ($\varphi = 27.7 \pm 11.0$ nm).

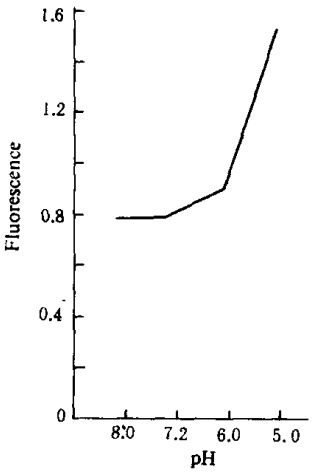


Fig. 2. pH-sensitivity of immunoliposomes assay.

pH-sensitive, it is stable in pH 8.0—7.0, but easy to release its contents in the slightly acidic environment of target cell plasma, bellow pH 6.0. Obviously, it is advantageous to killing target tumor cells.

### 2. *Thermotropic Phase Transition of the Immunoliposomes*

Fig. 3 shows that the thermotropic phase transition temperature of the immunoliposomes containing derivatized IgG is about 22°C (from stable bilayer phase to $H_{II}$ phase). When CT was incorporated, the temperature of the thermotropic phase transition changed to 35°C . This indicates that the immunoliposomes are more stable[6, 7]. It is interesting that CT can destroy biomembranes violently but not the membrane of the immunoliposomes. There are some hypotheses about the mechanism by which CT destroys biomembranes[9—12], including: (1) penetrating the phosphatide membrane directly; (2) suppressing the activity of $Na^+$, $K^+$-ATP enzymes; (3) transferring $Ca^{++}$ ions in the membrane; (4) increasing the permeability of membrane. Our experiments indicate that CT probably acts on proteins or enzymes in the biomembranes.

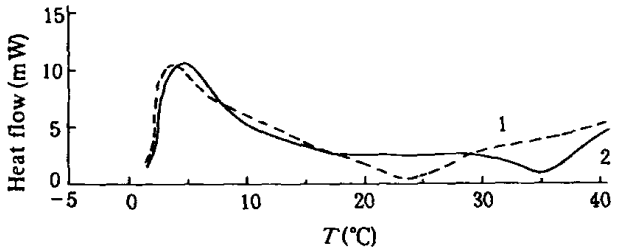


Fig. 3. Thermotropic phase transition of the immunoliposomes. ——, Immunoliposomes containing derivatized IgG and CT; – – –, immunoliposomes containing derivatized IgG.

### 3. *Specific Cytotoxicity of CT Encapsulated in Immunoliposomes*

Calcein-containing immunoliposomes were incubated with CEM or Raji cells. No detectable fluorescence was observed on the Raji cells. In contrast, CEM cells showed bright fluorescence in the cytoplasma and on the cell peripheries. Cells incubated with free calcein showed no uptake of dye. Our results suggest that the immunoliposomes bind specifically to

the target cells. Because pretreatment of CEM with free antibody could partly block the immunoliposome cell-killing, it demostrates that specificity of the immunoliposomes can be mediated by the antibody.

Treatment with the immunoliposomes containing CT after 8 h of culture showed that 78.4% of CEM cells were killed, while only 20.4% Raji cells were killed in the same condition (Fig. 4), it indicates that the immunoliposomes are highly cytotoxic to the human T-leukemic cells but not to antigen negative cells.

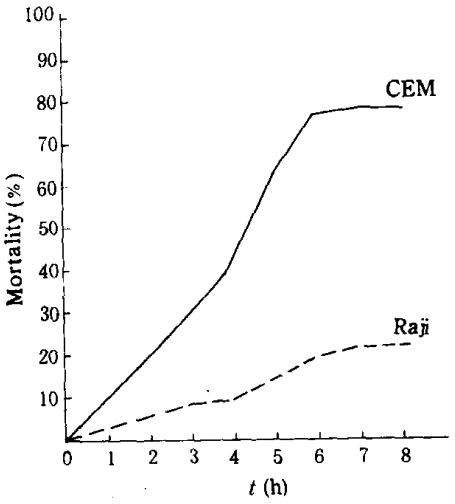


Fig. 4. Cytotoxicity of the immunoliposomes on CEM and Raji cell line.

Our previous work demonstrated that antibody-CT conjugate could specially kill target cells in vitro by using a special suppressing/reacting method[8]. This study has demonstrated the feasibility of using antibody-mediated pH-sensitive immunoliposomes as targeted carriers of CT. These immunoliposomes might be useful to invitro eradication of leukemic cells in autologous bone marrow transplantation to leukemic patients. It might also be useful to elimination of alloreactive T cells from bone marrow grafts for graftversu-host disease praphylaxis in allogeneic bone marrow transplantation.

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