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| Title | A DNA Microarray-based Analysis of the Host Response to a Nonviral Gene Carrier: A Strategy for Improving the Immune Response |
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Supplementary Materials and Methods

Materials

Male ICR mice (5-6 weeks old) were purchased from CLEA (Tokyo, Japan). ELISA assay kits of Quantikine Immunoassay mouse INF- γ was purchased from R&D systems (Minneapolis, MN, USA). [^3H]cholesteryl hexadecyl ether (CHE), Soluene-350 and Hionic Flour were purchased from Perkin-Elmer Life Sciences, Japan (Tokyo, Japan). Other materials were purchased as described Materials and Methods section. Transaminase CII-test WAKO was obtained from Wako (Osaka, Japan).

Methods

Determination of serum biochemical value and cytokine

ALT levels in serum were measured with test kits, and IL-6, IFN- γ , TNF- α , and IFN- α levels in serum were determined with ELISA kits according to the manufacturer's instructions.

Accumulation of systemically administered NPs in the spleen

A lipid film was prepared in a glass test tube by evaporating a chloroform solution of lipids, containing DOTAP, DOPE and cholesterol (300 nmol total lipids in 3:4:3 molar

ratio). [³H]CHE was also added in lipid solution as a tracer (Stein Y, Halperin G, Stein O. Biological stability of [³H]cholesteryl oleyl ether in cultured fibroblasts and intact rat. *FEBS Lett* 1980; **111**: 104-106.). The modification of PEG-DSPE and chol-GALA and subsequent encapsulation of pDNA/PEI complex were performed as described in Materials and Methods section. NPs labeled with [³H]CHE were intravenously injected at a normal pressure (25 µg pDNA/mouse). At 2 hr after i.v. injection, spleen was collected and solubilized in 1 ml of Soluene-350 at 42°C for 12 hr. The radioactivities were determined by liquid scintillation counting, after adding 10 ml of Hionic Flour.