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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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Citation	Molecular Therapy, 19(8), 1487-1498 https://doi.org/10.1038/mt.2011.24
Issue Date	2011-08
Doc URL	http://hdl.handle.net/2115/48250
Type	article (author version)
Additional Information	There are other files related to this item in HUSCAP. Check the above URL.
File Information	Mol Ther Hatakeyama. Revised Supplementary Materials and Methods.pdf (Supplementary Materials and Methods)

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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). [^{3}H]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). [^{3}H]CHE was also added in lipid solution as a tracer (Stein Y, Halperin G, Stein O. Biological stability of [^{3}H]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 [^{3}H]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.