Efficient Gene Transfer into Macrophages and Dendritic Cells by in Vivo Gene Delivery with Mannosylated Lipoplex via the Intraperitoneal Route

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ABSTRACT

In this study, we developed an antigen-presenting cell (APC)-selective intraperitoneal (i.p.) gene delivery system with mannosylated cationic liposomes (Man-liposomes)/plasmid DNA complex (Man-lipoplex). An in vitro study using cultured peritoneal macrophages demonstrated that Man-liposomes could transfect luciferase-encoding plasmid DNA (pCMV-Luc) more efficiently than cationic liposomes via a mannose receptor-mediated mechanism. In vivo gene transfection studies revealed that Man-lipoplex showed a higher gene expression in the liver, spleen, peritoneal exuded cells, and mesenteric lymph nodes than cationic liposomes/plasmid DNA complex (lipoplex) or naked pCMV-Luc after i.p. administration, and this gene expression lasted for at least 24 h. The transfection activity of Man-lipoplex after i.p. administration was significantly higher than that after i.v. gene delivery with the Man-liposomes we developed previously, indicating that gene delivery via the i.p. route seems to be an efficient approach for in vivo gene delivery to APCs. Furthermore, it was demonstrated that Man-lipoplex could enhance gene expression in both F4/80<sup>+</sup> and CD11c<sup>+</sup> cells in the spleen. These results show that gene delivery with Man-liposomes via the i.p. route could be an effective approach for APC-selective gene transfection.

The success of in vivo gene therapy relies on the development of a vector that achieves target cell-specific, efficient, and prolonged transgene expression after its application. Gene delivery to antigen-presenting cells (APCs) including macrophages and dendritic cells is of great therapeutic potential for DNA vaccination (Porgador et al., 1998; Akbari et al., 1999). Despite the high transfection efficiency of viral vectors, safety concerns have been raised in clinical trials because of their highly toxic nature (Arthur et al., 1997; Raper et al., 2003). Nonviral vectors, such as cationic liposomes (Lee and Huang, 1997; Hashida et al., 2005) and polymers (Dufes et al., 2005; Neu et al., 2005), are considered to be less toxic, less immunogenic, and easier to prepare than viral vectors and are, therefore, more attractive vectors for clinical applications. Despite their usefulness, nonviral vectors lack cell specificity in their delivery (Kawakami et al., 2005). It is well known that mannose receptors are expressed exclusively on APCs, including dendritic cells, and, so, introduction of mannose residues to cationic liposome formulations could provide an ideal DNA vaccine therapy.

Recently, we developed a novel mannosylated cationic cholesterol derivative, (cholesten-5-yloxy-N-[1-2,3-dioloyloxy]propyl]-N,N,N-trimethylammonium chloride (DOTMA), N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride; DOPE, diphosphatidylethanolamine; FBS, fetal bovine serum; RT, reverse transcription; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; PEC, peritoneal exudate cell.) for the preparation of mannosylated cationic liposomes (Man-liposomes). Previously, we succeeded in delivering the reporter gene, firefly luciferase (pCMV-Luc), selectively to APCs via mannose receptor-mediated endocytosis after i.v. administration of Man-liposomes/plasmid DNA complex (Man-lipoplex) (Kawakami et al., 2000). However, lipoplex interacted with erythrocytes, which reduces the transfection efficacy after intravascular administration (Hattori et al., 2004; Fumoto et al., 2005), because of its cationic nature.

The transfection efficiency to APCs under in vivo conditions was significantly higher when MAN-lipoplex was administered via the i.p. route, compared to i.v. administration. This suggests that the i.p. route may be an efficient approach for in vivo gene delivery to APCs. The use of mannosylated liposomes may provide an effective approach for the treatment of diseases associated with APC dysfunction.
tions seems to rely on not only the ability of the gene vector but also the administration route since APCs are widely distributed in the body and accessibility to APCs depends largely on the administration route chosen. Intraperitoneal administration has some advantages for transfection efficacy to APCs by Man-lipoplex because of 1) high accessibility to APCs in the peritoneal cavity and lymph nodes, 2) few bio-components that reduce transfection activity, and 3) high capacity of the lipoplex solution. Taking these factors into consideration, i.p. administered Man-lipoplex could achieve enhanced long-term gene expression in APCs. However, few reports are available on the transfection efficacy in APCs with Man-lipoplex after i.p. administration.

In this study, we examined the transfection efficacy to APCs including dendritic cells after i.p. administration of Man-lipoplex into mice. To evaluate the effect of mannosylation of the lipoplex on the transfection efficacy in APCs, conventional lipoplex was also administered i.p.

**Materials and Methods**

**Materials.** Cholesteryl chloroformate, HEPES, concanavalin A, G418, and immunogoldin G were obtained from Sigma Chemicals Inc. (St. Louis, MO). N-1-(2,3-Dioleoyloxy)propyl-V-N, N-trimethyl-ammonium chloride (DOTMA) and N-(4-aminobutyl) carbamic acid tert-butyl ester were obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). Diphosphatidylethanolamine (DOPE) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). pcDNA3.1, fetal bovine serum (FBS), and Opti-MEM I were obtained from Invitrogen (Carlsbad, CA). Anti-F4/80 rat IgG antibody-labeled magnetic beads were purchased from Serotec (Oxford, UK). Anti-rat IgG antibody-labeled magnetic beads and anti-mouse CD11c antibody-labeled magnetic beads were purchased from Miltenyi Biotec Inc. (Auburn, CA). A nucleic acid purification kit, MagExtractor-RNA, was purchased from Toyobo Co., Ltd. (Osaka, Japan). A first-strand cDNA synthesis kit for RT-PCR, Lightcycler Faststart DNA master hybridization probes, and Lightcycler-Primer/Probes set for mouse β-actin were purchased from Roche Diagnostics (Indianapolis, IN). Primers/probes for firefly luciferase were purchased from Nihon Gene Research Labs Inc. (Sendai, Japan). All other chemicals were of the highest purity available.

**Animals.** Female ICR mice (4–5 week old) were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). All animal experiments were carried out in accordance with the Principles of Laboratory Animal Care as adopted and promulgated by the National Institutes of Health (publication 85-23, revised 1985) and the guidelines for animal experiments of Kyoto University.

**Synthesis of Man-C4-Chol.** Man-C4-Chol was synthesized as described previously (Kawakami et al., 2000). In brief, N-(4-aminobutyl)-(cholest-5-enoyloxyl)formamide was synthesized from cholesteryl chloroformate and N-(4-aminobutyl)carbamic acid tert-butyl ester. The N-(4-aminobutyl)-(cholest-5-enoyloxyl)formamide was reacted with 5 Eq of 2-imino-2-methoxyethyl-1-thiomannoside (Lee et al., 1976) in pyridine containing 1.1 Eq of triethylamine for 24 h. After evaporation of the reaction mixture in vacuo, the resultant material was suspended in water and dialyzed against water for 48 h and then lyophilized.

**Preparation of Cationic Liposomes and Man-Liposomes.** For the i.p. administration, DOTMA, cholesterol, and Man-C4-Chol were mixed in chloroform at a molar ratio of 2:1:1 and 1:1:0 to prepare Man-liposomes and cationic liposomes, respectively. For i.v. administration, Man-C4-Chol was mixed with DOPE in chloroform at a molar ratio of 3:2 to prepare Man-liposomes (i.v.) (Kawakami et al., 2000; Yamada et al., 2004). The mixture then was dried, vacuum-desiccated, and resuspended in sterile 20 mM HEPES buffer, pH 7.8, or 5% dextrose solution in a sterile test tube for in vitro and in vivo experiments, respectively. After hydration, the dispersion was sonicated for 5 min in a bath sonicator to produce liposomes and then sterilized by passing through a 0.45-μm filter (Nihon-Millipore Ltd., Tokyo, Japan).

**Preparation of Lipoplex or Man-Lipoplex for in Vitro Study.** Equal volumes of pCMV-Luc and stock liposome solution were diluted with Opti-MEM I in 15-ml Falcon tubes. Then, pCMV-Luc solution was added rapidly to the surface of the liposome solution at a charge ratio of 1:0.2:3 (–/-+) using a micropipette (Pipetman Milipore, Villier-le Bel, France), and the mixture was agitated rapidly by pumping it up and down twice in the pipette tip.

**Harvesting and Culture of Mouse Peritoneal Macrophages.** Elicited macrophages were harvested from ICR mice 4 days after i.p. injection of 1 ml of 2.9% thioglycolate medium (Nissui, Tokyo, Japan). The washed cells were suspended in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, penicillin G (100 U/ml), and streptomycin (100 μg/ml) and were plated on 12-well culture plates at a density of 5 × 10^6 cells/3.8 cm^2. After incubation for 24 h at 37°C in 5% CO2-95% air, nonadherent cells were washed off with culture medium, and cells were cultivated for another 48 h.

**Cellular Association Study in Cultured Peritoneal Macrophages.** The macrophages were plated on a 12-well cluster dish at a density of 5 × 10^6 cells/3.8 cm^2. The culture medium was replaced with an equivalent volume of Hanks’ balanced salt solution containing 1 kBq/ml [32P]plasmid DNA, 0.5 μg/ml plasmid DNA, and cationic liposomes. After incubation for given time periods, the solution was immediately removed by aspiration, the cells were washed five times with ice-cold Hanks’ balanced salt solution buffer and solubilized in 1 ml of 0.3 N NaOH solution with 10% Triton X-100. The radioactivity was measured by liquid scintillation counting (LSC; 500; Beckman, Tokyo, Japan), and the protein content was determined using a Dojindo Protein Quantification Kit (Dojindo Molecular Technologies, Inc., Gaithersburg, MD). The effect of the copresence of mannoside was determined in the same system.

**In Vitro Transfection Study in Cultured Peritoneal Macrophages.** Macrophages were seeded in 12-well plates at a density 5 × 10^6 cells/3.8 cm^2 in RPMI 1640 supplemented with 10% FBS. After 3 days in culture, the culture medium was replaced with Opti-MEM I containing 0.5 μg/ml plasmid DNA and cationic liposomes. Six hours later, the incubation medium was replaced again with RPMI 1640 supplemented with 10% FBS and incubated for an additional 18 h. Then, the cells were scrapped off and suspended in 200 μl of pH 7.4 phosphate-buffered saline (PBS), and 100 μl of cell suspension was subjected to three cycles of freezing (liquid N2 for 3 min) and thawing (37°C for 3 min), followed by centrifugation at 10,000g for 3 min. The supernatants were stored at −20°C until the luciferase assay was performed; 10 μl of supernatant was mixed with 100 μl of luciferase assay buffer (Pica gene; Toyo Ink Co., Ltd., Tokyo, Japan), and the light production was immediately measured in a luminometer (Lumat LB 9507; EG&G Berthold, Bad Wildbad, Germany). The activity is indicated as the relative light units per milligram of protein. The protein content of the cell suspension in PBS was determined with a Protein Quantification Kit (Dojindo Molecular Technologies, Inc.).

**Preparation of Lipoplex or Man-Lipoplex for in Vivo Study.** All lipoplexes for in vivo experiments were prepared under the optimal conditions for cell-selective gene transfection as reported previously (Kawakami et al., 2002). In brief, pCMV-Luc (100 μg) and stock liposome solution were diluted to 500 μl with 5% dextrose in 15-ml tubes. Then, pCMV-Luc solution was added rapidly to the surface of the liposome solution using a micropipette, and the mixture was agitated rapidly by pumping it up and down twice in the pipette tip. The mean particle size of the lipoplexes was measured by dynamic light scattering spectrophotometry (LS-900; Otsuka Electronics Co., Ltd., Osaka, Japan). The ζ-potential of the lipoplexes was measured by the laser Doppler electrophoresis method with ζ-sizer Nano ZS (Malvern Instruments, Ltd., Worcestershire, UK).

**In Vivo Gene Expression Study in Mice.** The lipoplexes were given to 5-week-old ICR mice using a 27-gauge syringe needle at a
dose of 100 μg of plasmid DNA. At predetermined times after administration, mice were sacrificed, and the peritoneal cavity was washed with 1 ml of saline to collect the peritoneally exuded cells (PECs). Then, the liver, spleen, and mesenteric lymph nodes were harvested. The organs were washed twice with ice-cold saline and homogenized with lysis buffer (0.05% Triton X-100, 2 mM EDTA, and 0.1 M Tris, pH 7.8). The lysis buffer was added in a weight ratio of 5 μl/mg for liver samples or 4 μl/mg for other organ samples. PECs were resuspended in lysis buffer (400 μl) after centrifugation (4000g for 5 min at 4°C). After three cycles of freezing and thawing, the homogenates were centrifuged at 10,000g for 10 min at 4°C and then a 20-μl supernatant was used to determine the luciferase activity using a luminometer (Lumat LB9507; EG&G Berthold). The protein concentration of each tissue extract was determined using a Dojindo Protein Quantification Kit (Dojindo Molecular Technologies, Inc.). Luciferase activity in each organ was normalized to relative light units per milligram of extracted protein.

Quantification of Luciferase mRNA

Aliquots of clodronate-containing liposomes (400 μl) were administered i.v. via the tail vein or i.p. to ICR mice. Then, 48 h after administration of clodronate-containing liposomes, a gene transfection experiment was performed as described under In Vivo Gene Expression Study in Mice.

Statistical Analysis

Statistical analysis was performed using Student’s paired t test for two groups and the Turkey-Kramer test for multiple comparisons between groups. P < 0.05 was considered to be indicative of statistical significance.

Results

Particle Sizes and ζ-Potential of Lipoplex or Man-Lipoplex. To investigate the physicochemical properties of the lipoplexes, their particle size and ζ-potential were evaluated. Both lipoplexes showed a clear-cut distribution pattern, and the mean particle sizes of the Man-lipoplex and lipoplex were 126 ± 4.67 and 120 ± 7.03 nm (n = 3), respectively. ζ-Potential analysis showed that the ζ-potential of the Man-lipoplex and the lipoplex was 64.8 ± 0.73 and 65.6 ± 0.99 mV (n = 3), respectively. These results show that there was no significant difference in physicochemical properties between the two lipoplexes.

![Cellular association of lipoplex or Man-lipoplex in cultured macrophages](https://example.com/image.png)

**Fig. 1.** Cellular association of lipoplex or Man-lipoplex in cultured macrophages. a, cellular association time course of 32P-labeled Man-lipoplex (●) and lipoplex (○) in macrophages at 37°C. b, cellular association of 32P-labeled pCMV-Luc complexed with liposomes in the absence (●) or presence (○) of 1 mg/ml mannan in culture medium. Each value represents the mean ± S.D. (n = 3). Statistically significant differences from lipoplex or absence of mannan (*, P < 0.05).
Cellular Association of Lipoplex or Man-Lipoplex in Cultured Peritoneal Macrophages. To evaluate the potency of Man-lipoplex for targeted delivery to APCs, the uptakes of the Man-lipoplex and lipoplex were compared. In this study, we used cultured mouse peritoneal macrophages as APCs that expresses the mannose receptor. The Man-lipoplex showed significantly higher association to macrophages than lipoplex (Fig. 1a), and this was reduced in the presence of an excess of mannan (Fig. 1b).

Transfection Activity of Lipoplex or Man-Lipoplex in Cultured Peritoneal Macrophages. We next investigated the transfection activity of the Man-lipoplex to macrophages. As shown in Fig. 2a, the Man-lipoplex showed higher gene expression than lipoplex. The transfection activity of the Man-lipoplex was significantly reduced in the presence of an excess of mannan (Fig. 2b).

Transfection Activity of Lipoplex or Man-Lipoplex via Intraperitoneal Administration in Mice. After i.p. administration, Man-lipoplex showed higher transfection activity than lipoplex in the liver, spleen, PECs, and mesenteric lymph nodes (Fig. 3). As shown in Fig. 4, Man-lipoplex showed significantly higher gene expression than lipoplex and this gene expression lasted for more than 48 h.

The transfection activity after i.p. administration of Man-lipoplex showed significantly higher gene expression than after i.v. administration of Man-lipoplex both in the liver (Fig. 5a) and in the spleen (Fig. 5b). Furthermore, whereas the transfection activity was reduced 24 h after i.v. administration of Man-lipoplex, the transfection activity lasted for more than 48 h after i.p. administration of Man-lipoplex.

Transfection Activity in Macrophages and Dendritic Cells after Intraperitoneal Administration of Lipoplex or Man-Lipoplex in Mice. To clarify the transfection activity of Man-liposomes to APCs, we further investigated the transfection activity to macrophages and dendritic cells in the spleen as a representative of lymphoid tissue after i.p. administration. As shown in Fig. 6, Man-lipoplex induced significantly higher luciferase mRNA expression than lipoplex in both F4/80<sup>+</sup> cells (Fig. 6a) and CD11c<sup>+</sup> cells (Fig. 6b). These results demonstrated that Man-lipoplex enhances gene expression in specific cell types, such as macrophages and dendritic cells in the lymphoid tissue after i.p. administration.

Effect of Macrophage Depletion on Transfection Activity of Lipoplex or Man-Lipoplex via i.p. Administration in Mice. To investigate the contribution of the APCs in the transfection activity, we depleted peritoneal macrophages by i.p. injection of an anti-F4/80 antibody before i.p. injection of lipoplex or Man-lipoplex. As shown in Fig. 7, the transfection activity was significantly reduced after macrophage depletion compared to that in wild-type mice (Fig. 7a). Furthermore, the transfection activity was significantly lower in F4/80<sup>-</sup> mice than in F4/80<sup>+</sup> mice (Fig. 7b). These results suggest that macrophages are essential for the transfection activity of lipoplex or Man-lipoplex after i.p. administration.

Fig. 2. Transfection activity of lipoplex or Man-lipoplex in cultured macrophages. a, pcMV-Luc (0.5 μg/ml) was complexed with cationic liposomes or Man-liposomes. b, effect of the copresence of 1 mg/ml mannan on the transfection activity in macrophages. Cells were transfected with lipoplexes in the presence (□) and absence (▲) of 1 mg/ml mannan. Each value represents the mean ± S.D. (n = 4). Statistically significant differences from lipoplex or absence of mannan (*, P < 0.05). RLU, relative light unit.

Fig. 3. Transfection activity in liver, spleen, greater omentum, PECs, and mesenteric lymph node 6 h after i.p. administration of lipoplex (□) or Man-lipoplex (▲). pcMV-Luc (100 μg) was complexed with cationic liposomes or Man-liposomes. Transfection activity was determined 6 h after i.p. administration. Each value represents the mean ± S.D. (n = 3). Statistically significant differences compared with lipoplex (+, P < 0.05).

Fig. 4. Transfection activity time course in liver (a), PECs (b), spleen (c), and mesenteric lymph node (d) after i.p. administration of lipoplex (□) or Man-lipoplex (▲). pcMV-Luc (100 μg) was complexed with cationic liposomes or Man-liposomes. The transfection activity was determined at predetermined times after i.p. administration. Each value represents the mean ± S.D. (n = 3). Statistically significant differences compared with lipoplex (+, P < 0.05). RLU, relative light unit.
blood vessels to the transfection activity, the transfection activity of Man-lipoplex was examined in two kinds of macrophage-depleted mice. One group was mice in which macrophages accessible from blood vessel were depleted by i.v. administration of clodronate-incorporating liposomes. Another group was mice, in which macrophages in the bloodstream, peritoneal cavity, lymph nodes, liver, and spleen were depleted by i.p. administration of clodronate-incorporating liposomes. As shown in Fig. 7b, the transfection activity of Man-lipoplex was significantly reduced in various organs whose macrophages in the peritoneal cavity, lymph nodes, blood vessels, liver, and spleen had been depleted (Fig. 7b). On the other hand, only depletion of macrophages accessible from blood vessels did not reduce the transfection activity of Man-lipoplex (Fig. 7a). These results strongly suggested that Man-lipoplex could not achieve gene transfection to APCs in the bloodstream or APCs accessible from the bloodstream.

**Discussion**

APCs are an attractive target for the gene therapy of several diseases such as DNA vaccine therapy, cancer, human immunodeficiency virus, and others. Recently, we developed Man-C4-Chol with bifunctional properties of plasmid DNA binding via electrostatic interaction and a high affinity for APCs via their mannose receptors (Kawakami et al., 2000). Because Man-C4-Chol possesses an imino group for binding to plasmid DNA via electrostatic interaction, many mannose units could be introduced on the liposomal surface without loss of the binding affinity to plasmid DNA. These promising properties of our Man-lipoplex enable APC-selective gene transfer under in vivo conditions (Kawakami et al., 2000; Hattori et al., 2005, 2006). In the present study, we developed Man-liposomes for APC-selective gene delivery system via the i.p. route and demonstrated that our system achieved a higher and more sustained gene expression than the APC-selective gene delivery system given by i.v. administration that we had developed previously. This in vivo gene delivery system to APCs offers a potent approach to gene therapy.

To demonstrate the mannose receptor-mediated gene transfection of the Man-lipoplex to APCs, its transfection characteristics in APCs were evaluated in both in vitro and in vivo experiments. In cultured macrophages, the Man-lipoplex showed significantly higher uptake and transfection activity than the lipoplex, and this was reduced in the presence of mannan, suggesting that the Man-lipoplex could achieve efficient gene transfection via mannose receptor-mediated endocytosis (Figs. 1 and 2). To clarify the in vivo gene expression of Man-lipoplex to APCs in tissues, we examined the gene expression in macrophages and dendritic cells in the spleen, one of the lymphoid tissues. After i.p. administration of Man-lipoplex, the gene expression in the liver, spleen, and peritoneal exudate cells, which contain a large number of APCs, was significantly higher than that of lipoplex (Figs. 3 and 4). Furthermore, both F4/80<sup>+</sup> cells and CD11c<sup>+</sup> cells were separated to examine the cells transfected by Man-lipoplex. As shown in Fig. 6, Man-lipoplex showed higher gene expression in both F4/80<sup>+</sup> cells and CD11c<sup>+</sup> cells, demonstrating that Man-lipoplex could induce higher gene expression in APCs in the lymphoid tissue. These observations provide evidence that there is efficient gene transfer into macrophages and dendritic cells by mannose receptors after i.p. administration of Man-lipoplex.

**Fig. 5.** Transfection activity time course in liver (a) and spleen (b) after i.v. administration of Man-lipoplex (○) and i.p. administration of Man-lipoplex (●). pCMV-Luc (100 μg) was complexed with each type of Man-liposomes. The transfection activity was determined at 6 h after i.p. administration. Each value represents the mean ± S.D. (n = 3). Statistically significant differences compared with i.v. administration of Man-lipoplex (*, P < 0.05). RLU, relative light unit.

**Fig. 6.** In vivo mRNA gene expression on F4/80<sup>+</sup> (a) cells and CD11c<sup>+</sup> cells (b) in the spleen after i.p. administration of Man-lipoplex. pCMV-Luc (100 μg) was complexed with cationic liposomes or Man-liposomes. Six hours after i.p. administration, luciferase mRNA and β-actin mRNA eluted from F4/80<sup>+</sup> or CD11c<sup>+</sup> cells were determined by quantitative RT-PCR. Each value represents the mean ± S.D. (n = 3–4). Statistically significant differences compared with the naked pCMV-Luc and lipoplex-injected group (**, P < 0.01).
We previously reported that the lipid composition was an important factor in APC-selective gene transfer for the i.v. administration of Man-lipoplex. Among the series of studies of Man-lipoplex, we have demonstrated that Man-lipoplex prepared by Man-C4-Chol/DOPE liposomes could achieve APC-selective gene transfer because a distribution study revealed that Man-lipoplex prepared by Man-C4-Chol/DOPE liposomes was eliminated more rapidly from the lung and accumulated to a significantly higher degree in the liver (Hattori et al., 2005). Man-lipoplex must pass through the lung capillaries to reach most of the APC-containing tissues (i.e., liver and spleen); therefore, escaping the lung capillaries is the most important step for APC-selective gene transfer after i.v. administration. In the case of the i.p. route, Man-lipoplex has direct access to APCs; therefore, the limiting step for APC-selective gene transfer is different. After i.p. administration, in fact, the gene expression of Man-lipoplex prepared by Man-C4-Chol/DOPE liposomes was >5- to 10-fold less than that of Man-lipoplex prepared by DOTMA/cholesterol/Man-C4-Chol liposomes (data not shown). This finding agreed well with our previous report describing the intraportal administration of Man-lipoplex (Kawakami et al., 2001). It has been reported that cholesterol-containing liposomes are more stable than DOPE-containing liposomes (Li et al., 1999; Sakurai et al., 2001) and, therefore, a stable formulation of Man-lipoplex (i.e., prepared with DOTMA/cholesterol/Man-C4-Chol liposomes) may be suitable for APC-selective gene transfer in the case of i.p. administration because of its direct accessibility to APCs. As shown in Fig. 5, intraperitoneally injected Man-lipoplex showed significantly higher gene expression than i.v. injected Man-lipoplex. Furthermore, the transfection activity after administration of Man-lipoplex lasted at least 48 h, whereas after i.v. administration the transfection activity disappears within 24 h. These results support our hypothesis that i.p. administration of Man-lipoplex should produce sustained gene expression because of the physiological characteristics of the peritoneal cavity.

In this study, we reported that gene delivery via the i.p. route could transfected gene to macrophages in not only the peritoneal cavity but also other organs, including the liver, spleen, and lymph nodes (Figs. 3 and 4). This result is consistent with other reports showing that spleen cell lymph nodes could be transfected by i.p. gene delivery with lipoplex (Philip et al., 1993; Fellowes et al., 2000). Although some researchers have discussed i.p. gene transfer by lipoplex (Ishii et al., 1997), the detailed mechanism remains unclear.

Possible explanations include 1) direct transfection to APCs via distribution of Man-lipoplex in the bloodstream and 2) migration of APCs after transfection in the peritoneal cavity. To investigate the transfection mechanism into APCs, the transfection activity in two kinds of APC-depleted mice were examined. In this study, we used macrophage-depletion techniques with clodronate-incorporating liposomes that has been widely used for studies, including immunology and gene therapy (Naito et al., 1996; Kuzmin et al., 1997; Van Rooijen et al., 1997; Van Rooijen and Van Kesteren-Hendrikx, 2002). After i.p. and i.v. administration of clodronate-incorporating liposomes, macrophages could be depleted according to the administration route (Van Rooijen and Sanders, 1994; Biewenga et al., 1995; Van Rooijen et al., 1997). Because Man-lipoplex could selectively transfected APCs by mannos recepto, transfection is difficult in the liver, spleen, greater omentum, and PECs without APCs. In fact, the transfection activity by Man-lipoplex was significantly reduced in mice in which macrophages in the peritoneal cavity, lymph nodes, blood vessels, liver, and spleen were depleted by the i.p. administration of clodronate-incorporating liposomes (Fig. 7b). On the other hand, this phenomenon did not occur in the mice whose macrophages were depleted only in blood vessels, liver, and spleen by i.v. administration of clodronate-incoroporating liposomes. These results suggest that gene transfection to the liver, spleen, greater omentum, and PECs does not occur via distribution of Man-lipoplex in the bloodstream.

Lymphoid organs, such as the lymph nodes and spleen, are attractive targets for DNA vaccine therapy (Cano et al., 2001; Maloy et al., 2001; Munoz-Montesino et al., 2004). In this study, we have demonstrated that i.p. administration of Man-lipoplex, which could efficiently transfected genes to APCs in the lymphoid tissue, could be a potent vector for DNA vaccine therapy after in vivo administration. For the clinical application of i.p. administration of Man-lipoplex, i.p. infusion of drugs such as cisplatin (Sabbatini et al., 2004) and paclitaxel (Gelderblom et al., 2000) as well as lipoplex (Madhusudan et al., 2004) has already been performed in clinical trials for ovarian cancer therapy. Regarding the i.p. administration method, implantable infusion pumps have been developed for a number of diseases (Hepp, 1994), and there has been remarkable progress in endoscopic and laparoscopic surgical techniques (Stellato, 1992). This progress in surgical techniques and devices could make i.p. administration of the Man-liposome formulation a conventional and feasible approach for future clinical applications.

To date, little has been reported on targeted in vivo gene
transfer to APCs. Because the barriers on gene expression between in vitro and in vivo methods are different, in vivo evaluation is very important. In this study, we have demonstrated that gene delivery with Man-lipoplex via the i.p. route is an efficient gene transfer method to macrophages and dendritic cells involving the transfection mechanism, transgene terms, and transfected organs and/or cells. These observations in vivo transfection characteristics are partly supported by our recent report describing enhanced DNA vaccine potency by Man-lipoplex after i.p. administration (Hattori et al., 2006).

In conclusion, we have demonstrated that i.p. administration of Man-lipoplex efficiently enhances gene expression in macrophages and dendritic cells. Although further investigation is needed to clarify the efficiency of this system in clinical practice, this information will be valuable for the development of gene therapy in which APCs are the prime target population, such as DNA vaccine therapy.

References


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