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Comparison of gene expression efficiency and innate immune response induced by Ad vector and lipoplex

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Abstract

Vectors for gene expression are the essential tools for both gene therapy and basic research. There are two groups of gene therapy vectors, viral and non-viral vectors. At present, toxicity triggered by vectors is one of the major concerns for clinical trials. In general, non-viral vectors, such as plasmid DNA–cationic liposome complex (lipoplex), are thought to be safer than viral vectors, such as adenovirus (Ad) vector, although lipoplex is less efficient in term of gene expression than the Ad vector. However, there has been no study directly comparing the gene expression efficiency and safety of viral and non-viral vectors. Here, we present evidence that the Ad vector shows much more efficient gene expression and is safer than lipoplex, at least with respect to the innate immune response. After being systemically administered to mice, the Ad vector showed a transduction efficiency that was 2 to 5 log orders higher than that of lipoplex, depending on the organ. On the other hand, surprisingly, the administration of lipoplex produced a greater amount of inflammatory cytokines such as interleukin-6, interleukin-12, and tumor necrosis factor-α than did the administration of the Ad vector, whereas a comparable level of hepatotoxicity was induced by these vectors. The production of inflammatory cytokines induced by the injection of lipoplex was reduced when the CpG motifs were removed completely from plasmid DNA. Thus, care should be taken to ensure the innate immune response induced by gene therapy vectors, especially lipoplex.

Keywords: Adenovirus vector; Lipoplex; Gene therapy; In vivo gene expression; Innate immunity

1. Introduction

Vectors for gene expression are requisite tools not only for gene therapy but also for basic research, such as the functional analysis of novel genes. The success of gene therapy is largely dependent on gene delivery vectors, which can be categorized into two groups, viral and non-viral vectors [1]. The viral vectors, such as the adenovirus (Ad) vector, have great advantages such as high-level gene expression in a broad range of tissue, but such vectors are thought to lack safety because they are based on a pathogenic virus [2,3]. Another problem is that some kinds of viral vectors have an oncogenic function. Retrovirus and lentivirus vectors, which can insert foreign genes into the host DNA in a random manner, sometimes leads to canceration [4]. On the other hand, non-viral vectors based on plasmid DNA are thought to be safe even though their transduction efficiency is low [2].

Viruses are highly developed agents specialized in infection and the transfer of genetic material to the cells of other organisms, because infecting the host cells is an essential stage in their life cycle. On the other hand, the immune systems of mammals have evolved to counterattack the efforts of viral pathogens [5]. One of the viral vectors, the Ad vector, has several advantages, including the ability to package relatively large-sized foreign DNA, the ease with which it can be produced, and broad cell tropism [5,6]. However, many studies have shown that systemic administration of Ad vectors immediately triggers the innate immune response to elicit an acute inflammation, such as occurs with the secretion of

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inflammatory cytokines and tissue injury [5,7,8]. Because of these problems with viral vectors, non-viral vectors have gained increasing attention recently [1]. Among the non-viral vectors, the lipoplex (complexes of cationic liposome/ plasmid DNA) is the most studied and represents the most promising approach for human clinical trials [2]. However, the utility of non-viral vectors is often limited because of their low level and narrow range of exogenous gene expression [9,10]. As in the case of viral vectors, the production of inflammatory cytokines and tissue damage have been reported to be induced by the systemic injection of lipoplex even though it contains no viral components [11–15]. It has been shown that the immunostimulatory CpG motifs present in plasmid DNA are responsible for a significant portion of this acute response [16]. Plasmid DNA and bacterial DNA contain a much higher frequency of unmethylated CpG motifs than does mammalian DNA [11,15–17]. Toll-like receptor (TLR) 9 has been identified as the receptor involved in the recognition of immunostimulatory CpG motifs [18]. TLR9 is a member of the family of TLRs, which play a critical role in innate immunity. Ten family members have been identified so far, and they appear to activate NF-κB, leading to the production of inflammatory cytokines [15,18,19].

Although both viral and non-viral vectors have pathogenic profiles, no study has directly compared the transduction efficiency, especially in vivo, and safety of these vectors. Thus, we performed a comparative study of the transduction efficiency and the level of production of inflammatory cytokines after systemic injection of the Ad vector or lipoplex into mice.

2. Materials and methods

2.1. Plasmids and adenovirus vectors

The plasmid vector, pCMVL1, and the Ad vector, Ad-L2, which were constructed to express luciferase under the transcriptional control of the cytomegalovirus (CMV) promoter and bovine growth hormone (BGH) polyadenylation signal, were described previously [20–22]. pCpG-mcs was purchased from Invivogen (San Diego, CA).

pCMVL1 and pCpG-mcs were amplified in DH5α and GT115, respectively, and isolated by using EndoFree Plasmid Mega Kit (QIAGEN, Valencia, CA). The concentration of lipopolysaccharide (LPS) in DNA solution was measured using the Limulus HS-F Single Test (Wako, Osaka, Japan). The amount of LPS in the DNA solution was <0.1 Endotoxin unit/ μg DNA, which is the amount endorsed by QIAGEN.

Ad-L2 was amplified in 293 cells; and purified by $CsCl_2$ gradient centrifugation; dialyzed with a solution containing 10 mM Tris (pH 7.5), 1 mM $MgCl_2$, and 10% glycerol; and stored in aliquots at −70 °C. Virus particle titer was measured spectrophotometrically as described previously [23].

2.2. Preparation of DOTAP/Chol liposome and lipoplex

DOTAP/Chol liposome and lipoplex were prepared by a modification of the method used by Li et al. [12]. Briefly, an appropriate amount of DOTAP (AVANTI Polar Lipids, Alabastar, Al) was mixed with cholesterol in chloroform at the molar ratio of 1:1. The organic solvent was evaporated to make the dried lipid film. The dried films were then hydrated in 5% dextrose solution under a 37 °C water bath to make liposome solution. The liposome solution was sonicated for 1–2 min before the lipoplex was prepared. To prepare the lipoplex, plasmid DNA was diluted with 5% dextrose, and then liposome solution was added to achieve a 9.8:1.0 weight ratio of DOTAP: DNA. The theoretical charge ratio (+/−) of the complex was 4.6. The mixture was incubated at room temperature for 10 min before injection.

2.3. In vivo gene transfer and luciferase assay

Female C57BL/6 mice (5–6 weeks) were purchased from Nippon SLC (Hamamatsu, Japan). A final volume of 200 μl of Ad vectors (1×10^{10} or 5×10^{10} vector particle (VP)/mouse) or lipoplex (5 or 25 μg of plasmid DNA/mouse) was injected intravenously via tail vain of each mouse. An appropriate length of time after the injection, the mice were given anesthesic by diethylether and their hearts, lungs, kidneys, livers and spleens were collected. The organs were then homogenized with lysis buffer (0.05% Triton X-100, 2 mM EDTA, 0.1 M Tris–HCl, pH 7.8). After being frozen and thawed, the homogenates were centrifuged at 15,000 ×g at 4 °C for 10 min, and the supernatants were collected. Luciferase activity in the supernatants was determined by using a luciferase assay system (PicaGene 5500; Toyo Inki, Tokyo, Japan). The protein content was measured with a Bio-Rad assay kit (Bio-Rad, Hercules, CA).

2.4. Cytokine measurement

An appropriate length of time after the injection, the mice were given anesthesic by diethylether and peripheral blood was collected via the inferior vena cava and fundus oculi. The peripheral blood was placed on ice for 2–3 h and then centrifuged at 15,000 rpm at 4 °C for 10 min to collect the blood serum. The cytokine concentration in serum was measured using an ELISA kit (IL-6 and IL-12p40; BD Biosciences, San Diego,CA, TNF-α; R & D Research Systems, Minneapolis, MN) according to the manufacturer's instructions. Alanine aminotransferase (ALT) activities of the blood serum were measured using the Transaminase CII-Test (Wako, Osaka, Japan).

2.5. Preparation of paraffin sections of liver

An appropriate length of time after the injection, the mice were given anesthesic by diethylether and their livers were collected. Each liver was washed, fixed in 10% formalin for 24 h at room temperature, and embedded in paraffin. After sectioning, the tissue was dewaxed in ethanol, rehydrated, and stained with hematoxylin and eosin. This process was commissioned to Applied Medical Research Laboratory (Osaka, Japan).

3. Results

3.1. Comparison of the transduction efficiency between Ad vector and lipoplex

To compare the transduction activity and safety of gene therapy vectors in vivo, we chose the Ad vector and DNA–liposome complex (lipoplex) as the viral vector and non-viral vector, respectively. We chose DOTAP/Chol as the liposome, because this has been used in many gene transduction studies in vivo, and its usability has been proven [2,10,24–26]. The mean diameter of the lipoplex, which was measured by Zetasizer (Malvern Instruments Ltd, United Kingdom) was about 350 nm (data not shown). Gel retardation assay showed that plasmid DNAs were indeed complexed with liposome (data not shown). To compare the in vivo transduction efficiency and the distribution of the gene expression from the Ad vector and lipoplex, we first analyzed the luciferase production in the organ after intravenous injection of the Ad vector (1×10^{10} or 5×10^{10} VP/mouse) or lipoplex (5 or 25 μg of plasmid DNA/mouse). The dose of the vectors injected in the present study was used in previous reports and was determined to be a limited dose that would not cause sudden death or raising of the hair [2,12]. High levels of luciferase production were obtained as a result of the injection of the Ad vector in all organs examined. On the other hand, lipoplex-mediated luciferase production was detected only in the heart and lung (Fig. 1). Luciferase production in lipoplex-injected mice was 10^{-5} to 10^{-2} lower than in Ad vector-injected mice in all organs. The luciferase production obtained from mice that were injected with lipoplex was approximately the same or slightly lower than has been shown in previous reports [2,25,27]. This difference might result from the differences in the mouse strains and liposome compositions used in the experiments. We also determined the duration of luciferase production after the intravenous injection

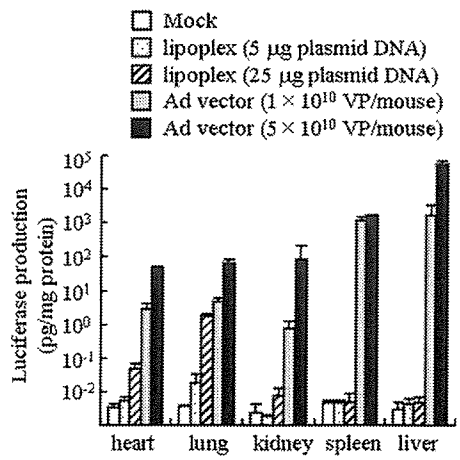

Fig. 1. Luciferase production in various organs after intravenous injection of the Ad vector or lipoplex. A final volume of 200 μl of Ad vectors (1×10^{10} or 5×10^{10} VP/mouse; gray and black bar, respectively) or lipoplex (5 or 25 μg of plasmid DNA; dotted and slashed bar, respectively) was injected intravenously into each mouse. Organs were collected from the mice 6 h following the injection, and luciferase activity and protein concentration were assayed. The white bar indicates mock treatment. Data are expressed as means±SD of 3–4 mice per group.

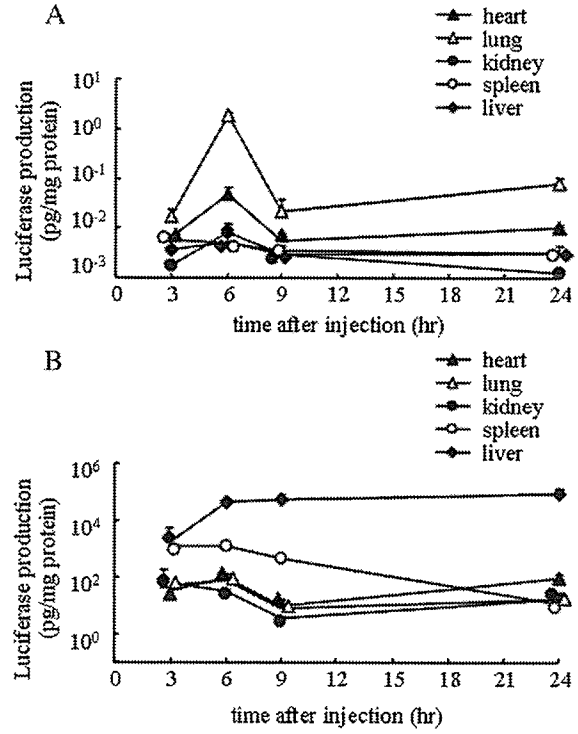

Fig. 2. Duration of the luciferase production. A final volume of 200 μl of lipoplex (25 μg of plasmid DNA; A) or Ad vectors (5×10^{10} VP/mouse; B) was injected intravenously into each mouse. After an appropriate length of time had passed following the injection, the heart (filled triangle), lung (opened triangle), spleen (opened circle), kidney (filled circle), and liver (filled diamond) were collected, and luciferase activity and protein concentration were measured. Data are expressed as means±SD of 3–4 mice per group.

of vectors (Fig. 2A and B). Luciferase production in all organs collected from mice that were injected with the Ad vector or lipoplex decreased in a time-dependent manner. It was striking that the livers collected from mice that were injected with high doses of the Ad vector maintained a high gene expression for 24 h (Fig. 2B). On the other hand, the gene expression of lipoplex decreased dramatically and reached levels similar to that shown in mock-treated mice in most organs after 9 h (Fig. 2A). Thus, we can conclude that the Ad vector can express a high level of foreign genes in a broad range of tissues.

3.2. Vector-triggered cytokine production

There are many indices of the side effects caused by the intravenous injection of vectors, such as canceration, tissue damage, innate and adaptive immune response, etc. In the present study, innate immune response was examined as an indicator of the side effects induced by the administration of vectors, since the induction of innate immune response by the Ad vector is the primary limiting factor in the use of the Ad vector [5]. To investigate the level of immune response induced by these vectors, we measured the serum concentration of various inflammatory cytokines, including IL-6, IL-12 and TNF-α. At 6 h following the intravenous injection, the IL-6 concentration in serum from lipoplex-injected mice was 2- to 4-fold higher than that in serum from the Ad vector-injected mice (Fig. 3a). A similar profile was obtained for the production of

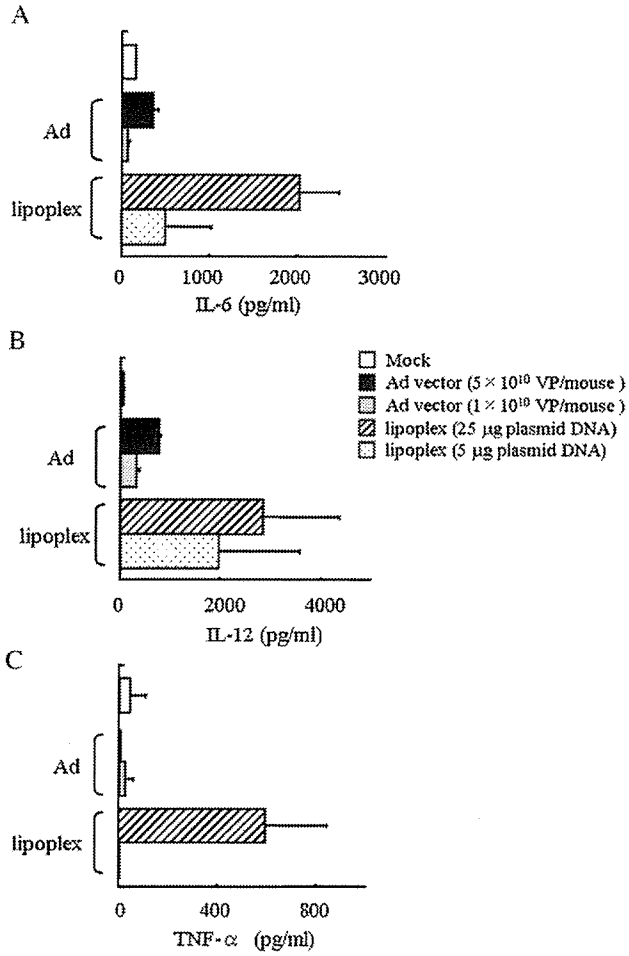

Fig. 3. Induction of various inflammatory cytokines by intravenous injection of the Ad vector or lipoplex. A final volume of 200 μl of Ad vectors (1×10^{10} or 5×10^{10} VP/mouse; gray and black bar, respectively) or lipoplex (5 or 25 μg of plasmid DNA; slashed and dotted bar, respectively) was injected intravenously into each mouse. Six hours after the injection, peripheral blood was collected, and the concentrations of IL-6, IL-12, and TNF-α were measured by ELISA. The white bar indicates mock treatment. Data are expressed as means ± SD of 3–4 mice per group.

IL-12 induced by these vectors (Fig. 3b). The level of cytokine production induced by these vectors was dose-dependent and returned to the basal level after 24 h post-injection (Fig. 6 shows the data for lipoplex; data not shown for the Ad vector). When a higher dose of vectors was injected, the serum concentration of TNF-α from lipoplex-injected mice was 2-orders higher than that from Ad vector-injected mice and control mice (Mock) (Fig. 3c). The serum concentration of TNF-α from the Ad vector-injected mice was similar to that from control mice (Mock). These results suggest that lipoplex induces the innate immune response more strongly than the Ad vector.

3.3. Hepatotoxicity caused by vector injection

The majority of intravenously injected Ad vector is sequestered by the liver, which in turn causes an inflammatory response characterized by acute transaminitis and vascular damage [7]. Systemic administration of lipoplex also leads to toxic effects in the liver [13]. We investigated the hepatic damage induced by the intravenous administration of the Ad vector and lipoplex. The hepatotoxicity induced by the vectors was compared by measuring the activity of alanine aminotransferase (ALT) in serum and by investigating the histopathological changes. There were no significant differences in the ALT activity between the sera from the Ad vector-injected mice (5×10^{10} VP/mouse) and the lipoplex-injected mice (both 5 and 25 μg plasmid-DNA/mouse) after 24 h post-injection (Fig. 4). The serum ALT activity in mice injected with 1×10^{10} VP of the Ad vector was the same as in the controls (i.e., the mock-treated levels). In the case of hepatic histological changes, hepatocytes from lipoplex-injected mice (25 μg plasmid-DNA/mouse) started to granulate 3 h after the injection, even though the cells from the Ad vector-injected mice (5×10^{10} VP/mouse) seemed to be normal (Fig. 5B and E). Six hours after injection, hepatocytes from the Ad vector-injected mice started to granulate similarly to those from lipoplex-injected mice (Fig. 5C and F). At 48 h after the injection, degranulation or denucleation occurred in hepatocytes from both the Ad vector- and lipoplex-injected mice (Fig. 5D and G). These results showed that both the Ad vector and lipoplex caused hepatotoxicity, especially at high doses, and that the liver damage caused by lipoplex, such as granulation and degranulation, started earlier than in the case of the Ad vector.

3.4. Effect of unmethylated CpG motifs in plasmid DNA on cytokine production

It has been reported that bacterial DNA induces innate immune response because it has a much higher frequency of unmethylated CpG dinucleotides than mammalian DNA [18,28]. The injection of plasmid DNA, which contains fewer CpG motifs, reduces the induction of inflammatory cytokines [13,26]. We examined the production of cytokines induced by the intravenous injection of lipoplex containing plasmid DNA without CpG motifs (non-CpG lipoplex), which is completely lacking in CpG motifs. In this experiment, only the level of inflammatory cytokine was examined, because luciferase-coding cDNA without CpG motifs was not obtained. As expected, the production of IL-6 induced by non-CpG lipoplex

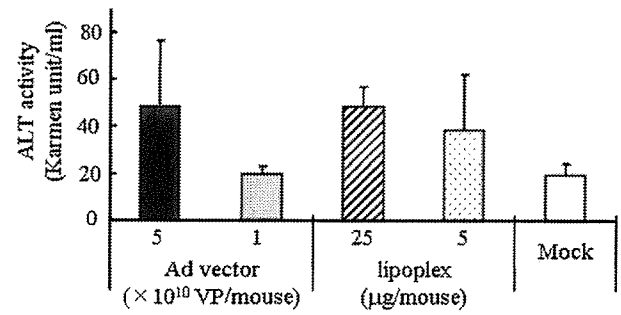

Fig. 4. Hepatotoxicity by intravenous injection of the Ad vector or lipoplex. A final volume of 200 μl of Ad vectors (1×10^{10} or 5×10^{10} VP/mouse; gray and black bar, respectively) or lipoplex (5 or 25 μg of plasmid DNA; slashed and dotted bar, respectively) was injected intravenously into each mouse. Twenty-four hours after the injection, peripheral blood was collected, and the ALT activity was measured. The white bar indicates mock treatment. Data are expressed as means ± SD of 3–4 mice per group.

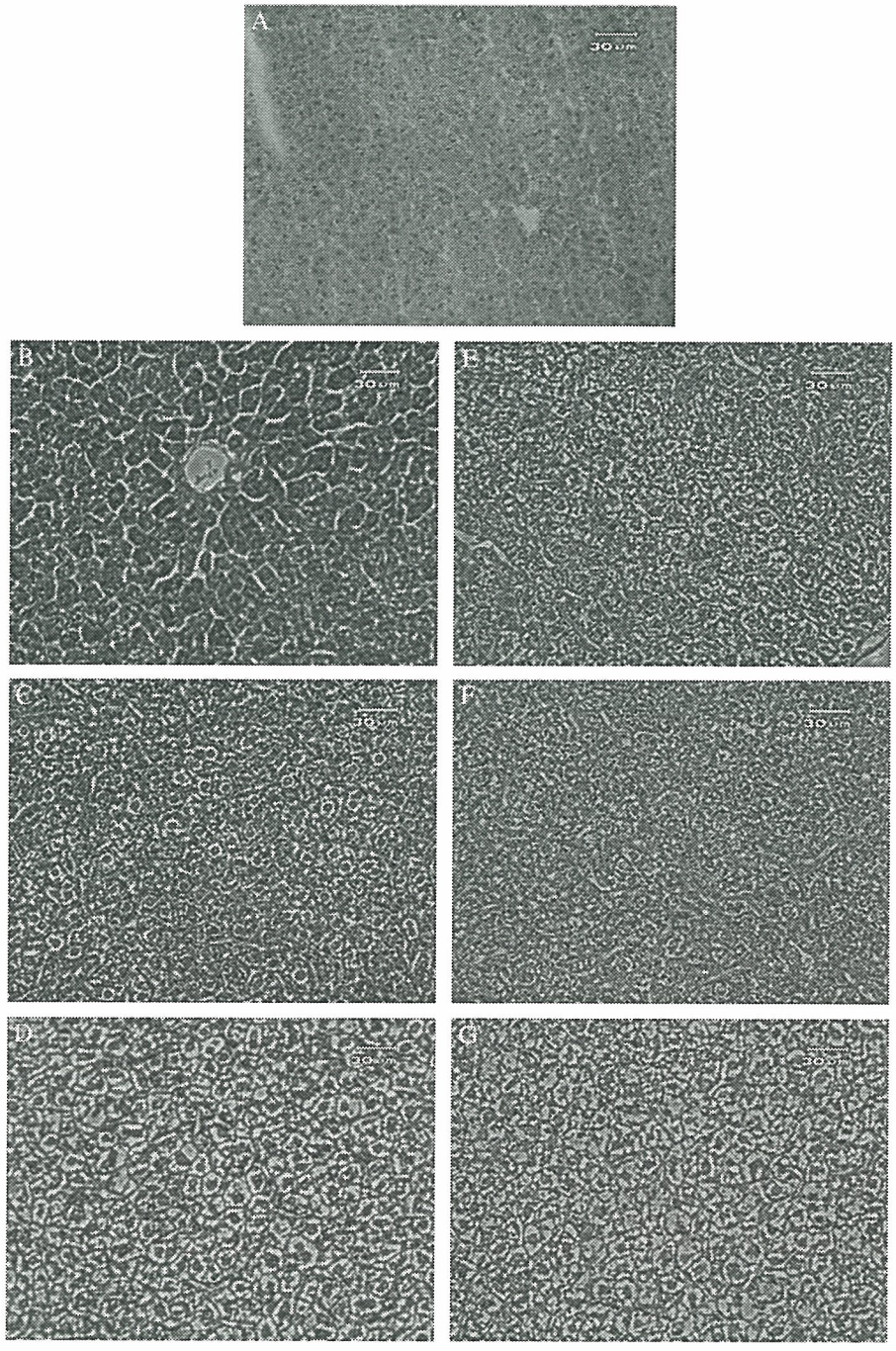

Fig. 5. Induction of acute liver failures by intravenous injection of the Ad vector or lipoplex. A final volume of 200 μl of Ad vectors (5×10^{10} VP/mouse; B–D), lipoplex (25 μg of plasmid DNA; E–G) or PBS (as Mock; A) was injected intravenously into each mouse. The livers were collected after 3 (B and E), 6 (C and F), and 48 (D and G) h following the injection, and paraffin sections were prepared. Each section was stained with hematoxylin and eosin.

was suppressed, but this complex still induced higher IL-6 production than the Ad vector (Figs. 3A and 6A). The injection of non-CpG lipoplex showed a different peak time of IL-6 production than in the case of CpG lipoplex injection. The level of producton of IL-12 and TNF-α induced by the injection of non-CpG lipoplex was lower than that induced by the injection of CpG lipoplex (Figs. 3B, C, 6B and C). The concentration of IL-12 in the serum from the non-CpG lipoplex-injected mice was lower than that in the serum from the Ad vector-injected mice. Unlike in the case of IL-6, non-CpG lipoplex showed the same profiles of IL-12 production as CpG-lipoplex. These results show that the removal of CpG motifs from the plasmid DNA in lipoplex could not completely suppress cytokine production, but there exists other mechanisms for suppressing immune response by lipoplex.

4. Discussion

A variety of viral and non-viral vectors have been developed for gene therapy [3,4,10]. At present, viral vectors dominate in

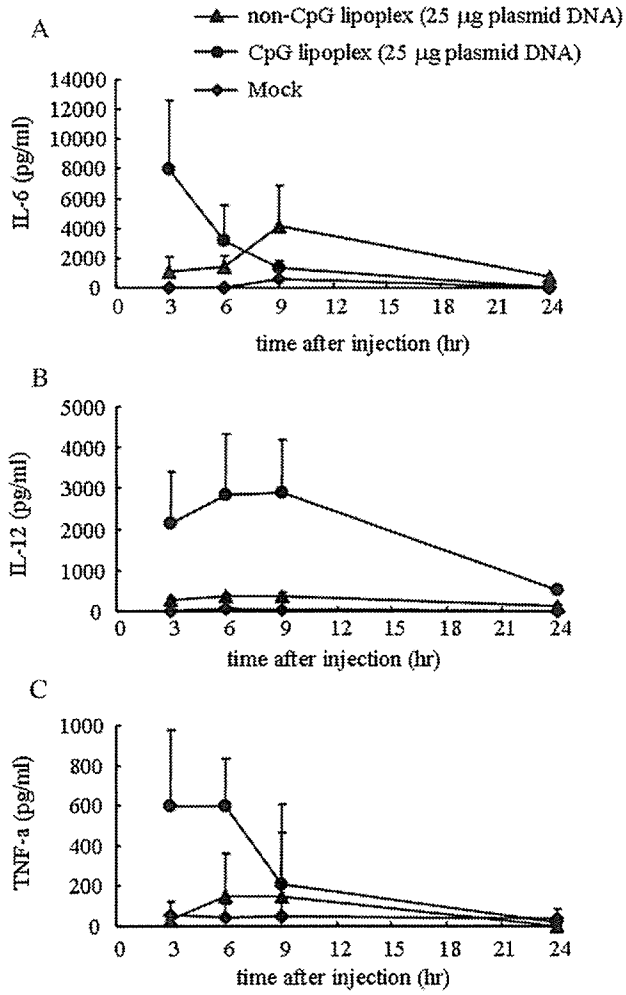

Fig. 6. Suppression of cytokine production by using non-CpG plasmid. CpG lipoplex containing pCMVL1 and non-CpG lipoplex containing pCpG-mcs were prepared as described in Material and methods. A final volume of 200 μl of CpG lipoplex (circle), non-CpG lipoplex (triangle) (25 μg of plasmid DNA) or 5% dextrose (as Mock; diamond) was injected intravenously into each mouse. Peripheral blood was collected at 3, 6, 9, 24, and 48 h after the injection, and the concentrations of IL-6 (A), IL-12 (B) and TNF-α (C) were measured by ELISA. Data are expressed as means±SD of 3–4 mice per group.

clinical trials because they are highly efficient in transducing cells. However, viral vectors are immunogenic and potentially mutagenic; thus, non-viral vectors have recently gained increasing attention [2]. In this study, we compared the in vivo usability and safety between a viral vector and a non-viral vector by examining the levels of reporter gene expression and inflammatory cytokine production (innate immune response). Our data revealed that the Ad vector, which is one of the major viral vectors used for gene therapy, is much more efficient for transduction and is also safer than lipoplex with respect to inflammatory cytokine production.

To achieve a therapeutic effect, gene therapy vectors should be able to deliver genes of interest to the designated target and to ensure their expression for an appropriate amount of time [29]. As shown in Fig. 1, the Ad vector mediated a high transduction efficiency in a wide range of organs, whereas lipoplex mediated a low level of expression only in the lung and heart. There might be two reasons for the difference in transduction efficiency between the Ad vector and lipoplex. First, the cells in most organs are composed of non-dividing cells. Ad vectors can infect both dividing and non-dividing cells, while lipoplex shows lower efficiency of gene transfer in non-dividing cells. The other reason is that there are different mechanisms for expressing transgenes between viral and non-viral vectors. The Ad vector has a unique system to be internalized into cells and to deliver foreign genes to the nucleus. Ad vectors first attach to the cell surface through an interaction between the fiber knob protein, one of the capsid proteins of Ad, and a high-affinity receptor, the coxsackievirus–adenovirus receptor (CAR). Then, another capsid protein, penton base, mediates virus internalization by receptor-mediated endocytosis. Following endosomal disruption, the partially uncoated virions traffic through the cytoplasm along microtubeles and reach the nuclear pore complex [30]. Interestingly, intravenously administered Ad vectors accumulate mainly in the liver, spleen, heart, lung and kidney in mice, even though these tissues may not necessarily have the highest level of CAR expression [29]. On the other hand, the specific receptor involved in the uptake of lipoplex remains unknown [10]. Lipoplex is taken up by an endocytosis mechanism, and the cytoplasmic delivery of DNA involves a fusion-related event, probably in the endosome compartment. One reason for the induction of limited transgene expression only in the lung and heart by lipoplex is the particle size of lipoplex, which is, on average, 350 nm, much bigger than that of the Ad vector (70–100 nm) [31,32]. Following intravenous administration, the larger lipoplex is known to lodge in the pulmonary capillaries [10,33]. The other possibility is that proteoglycans exposed at the cell surface mediate lipoplex-cell binding in the pulmonary vasculature [10,34].

For gene therapy to be successful, an appropriate amount of a therapeutic gene must be delivered into the target tissue without substantial toxicity [4]. We examined the innate immune response and hepatotoxicity induced by intravenous injection of the vectors and revealed that lipoplex was more highly immunogenic than the Ad vector, at least in terms of innate immune response. The innate immune response triggered by the Ad vector has been reported to be dose-dependent, occurs within 24 h after the injection, and is independent of viral or transgene transcription [5,30]. Our data are consistent with these observations. The production of cytokines induced by lipoplex is mainly due to the unmethylated CpG motifs in plasmid DNA [12]. Toll-like receptor 9 (TLR9) has been identified as the receptor involved in the recognition of unmethylated CpG motifs [18]. TLR9 is a member of the family of TLRs, which play a critical role in innate immunity, such as through the production of inflammatory cytokines [15,35]. Since the lipoplex is the complex of plasmid DNA and liposome, some plasmid DNAs are exposed outside the complex. When lipoplex enters the cells by endocytosis, the plasmid DNAs which are exposed outside the complex might be recognized by TLR9 expressed at the endosome. On the other hand, Ad vectors enters the cells with the genome (Ad DNA) encapsulated inside the capsid. TLR9 could not recognize Ad DNA, even though Ad DNA contains some CpG motifs, because Ad DNA is encapsulated by viral capsid in the

endosome, where TLR9 exists. A previous report showed that the absence of CpG signaling that occurs when $TLR9^{-/-}$ mouse is used greatly suppresses the innate immune response induced by lipoplex, but does not completely eliminate the acute toxic response, such as cytokine production [15]. Although this is not a complete remedy, one might predict that a completely non-CpG plasmid (CpG zero plasmid) vector would have an improved safety profile. We learned that the complete removal of CpG motifs from plasmid DNA could reduce cytokine production but the levels of inflammatory cytokine production were similar to that by the Ad vector, although it depended on the type of cytokines (Figs. 3 and 6). The production of inflammatory cytokines such as IL-6 and TNF-α could not be suppressed completely, although CpG motifs were removed from plasmid DNA in lipoplex. This result suggests that there might be different pathways to induce the production of inflammatory cytokines that are activated independently of the CpG motif. Un-identified sensor receptor(s), which recognize(s) foreign DNAs, might be involved in this phenomenon [36].

In this study, we used only one kind of liposome (DOTAP/chol). We could not draw the general conclusion that all types of lipoplex would show the same profile as DOTAP/Chol in inducing the innate immune response. However, the lipoplex that enters cells using the endocytic pathway might show the same tendency, because TLR9 is expressed at the endosome. Recently, many kinds of lipoplex and polyplex (complexes of cationic polymer/plasmid DNA) have been developed for the purpose of obtaining a higher transduction efficiency and suppressing the immune response [2,37,38]. Since TLR9 expression is in the endosomes of immunocompetent cells, a non-viral vector that can escape from endosomes quickly or that is not easily taken up by immune cells should be developed to eliminate the problem of the induction of the innate immune response.

Unlike the profile of cytokine production, the activity of ALT in sera from the lipoplex-injected mice was the same as that in sera from the Ad vector-injected mice. The histopathological changes in the liver in the Ad vector-or lipoplex-injected mice also showed a similar profile to each other. The mechanism of hepatotoxicity induced by those vectors is still unclear, but inflammatory cytokines might play a role in the hepatotoxicity.

It is commonly believed that non-viral vectors are safer to use in gene therapy than viral vectors. However, this study clearly showed that this would not be true, at least in innate immune response when the vectors are systemically injected. We hope that this present study will trigger a reconsideration of the safety of gene therapy vectors.

Acknowledgments

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References

[1] T. Niidome, L. Huang, Gene therapy progress and prospects: nonviral vectors, Gene Ther. 9 (24) (2002) 1647–1652.
[2] F. Liu, L.M. Shollenberger, L. Huang, Non-immunostimulatory nonviral vectors, FASEB J. 18 (14) (2004) 1779–1781.
[3] H. Mizuguchi, T. Hayakawa, Targeted adenovirus vectors, Hum. Gene Ther. 15 (11) (2004) 1034–1044.
[4] M.A. Kay, J.C. Glorioso, L. Naldini, Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics, Nat. Med. 7 (1) (2001) 33–40.
[5] D.A. Muruve, The innate immune response to adenovirus vectors, Hum. Gene Ther. 15 (12) (2004) 1157–1166.
[6] J.M. Wilson, Adenoviruses as gene-delivery vehicles, N. Engl. J. Med. 334 (18) (1996) 1185–1187.
[7] D.M. Shayakhmetov, A. Gaggar, S. Ni, Z.Y. Li, A. Lieber, Adenovirus binding to blood factors results in liver cell infection and hepatotoxicity, J. Virol. 79 (12) (2005) 7478–7491.
[8] Y. Zhang, N. Chirmule, G.P. Gao, R. Qian, M. Croyle, B. Joshi, J. Tazelaar, J.M. Wilson, Acute cytokine response to systemic adenoviral vectors in mice is mediated by dendritic cells and macrophages, Mol. Ther. 3 (5 Pt 1) (2001) 697–707.
[9] Y. Liu, D. Liggitt, W. Zhong, G. Tu, K. Gaensler, R. Debs, Cationic liposome-mediated intravenous gene delivery, J. Biol. Chem. 270 (42) (1995) 24864–24870.
[10] S.A. Audouy, L.F. de Leij, D. Hoekstra, G. Molema, In vivo characteristics of cationic liposomes as delivery vectors for gene therapy, Pharm. Res. 19 (11) (2002) 1599–1605.
[11] H. Hemmi, T. Kaisho, K. Takeda, S. Akira, The roles of Toll-like receptor 9, MyD88, and DNA-dependent protein kinase catalytic subunit in the effects of two distinct CpG DNAs on dendritic cell subsets, J. Immunol. 170 (6) (2003) 3059–3064.
[12] S. Li, S.P. Wu, M. Whitmore, E.J. Loeffert, L. Wang, S.C. Watkins, B.R. Pitt, L. Huang, Effect of immune response on gene transfer to the lung via systemic administration of cationic lipidic vectors, Am. J. Physiol. 276 (5 Pt 1) (1999) L796–L804.
[13] S. Loisel, C. Le Gall, L. Doucet, C. Ferec, V. Floch, Contribution of plasmid DNA to hepatotoxicity after systemic administration of lipoplexes, Hum. Gene Ther. 12 (6) (2001) 685–696.
[14] N.S. Yew, R.K. Scheule, Toxicity of cationic lipid–DNA complexes, Adv. Genet. 53 (2005) 189–214.
[15] H. Zhao, H. Hemmi, S. Akira, S.H. Cheng, R.K. Scheule, N.S. Yew, Contribution of Toll-like receptor 9 signaling to the acute inflammatory response to nonviral vectors, Mol. Ther. 9 (2) (2004) 241–248.
[16] R.K. Scheule, The role of CpG motifs in immunostimulation and gene therapy, Adv. Drug Deliv. Rev. 44 (2–3) (2000) 119–134.
[17] K. Yasuda, Y. Ogawa, I. Yamane, M. Nishikawa, Y. Takakura, Macrophage activation by a DNA/cationic liposome complex requires endosomal acidification and TLR9-dependent and-independent pathways, J. Leukoc. Biol. 77 (1) (2005) 71–79.
[18] H. Hemmi, O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda, S. Akira, A Toll-like receptor recognizes bacterial DNA, Nature 408 (6813) (2000) 740–745.
[19] T. Kawai, S. Akira, Innate immune recognition of viral infection, Nat. Immunol. 7 (2) (2006) 131–137.
[20] H. Mizuguchi, N. Koizumi, T. Hosono, N. Utoguchi, Y. Watanabe, M.A. Kay, T. Hayakawa, A simplified system for constructing recombinant adenoviral vectors containing heterologous peptides in the HI loop of their fiber knob, Gene Ther. 8 (9) (2001) 730–735.
[21] H. Mizuguchi, M.A. Kay, A simple method for constructing E1- and E1/E4-deleted recombinant adenoviral vectors, Hum. Gene Ther. 10 (12) (1999) 2013–2017.
[22] H. Mizuguchi, M.A. Kay, Efficient construction of a recombinant adenovirus vector by an improved in vitro ligation method, Hum. Gene Ther. 9 (17) (1998) 2577–2583.
[23] J.V. Maizel Jr., D.O. White, M.D. Scharff, The polypeptides of adenovirus. I. Evidence for multiple protein components in the virion and a comparison of types 2, 7A, and 12, Virology 36 (1) (1968) 115–125.
[24] L.S. Uyechi, L. Gagne, G. Thurston, F.C. Szoka Jr., Mechanism of lipoplex gene delivery in mouse lung: binding and internalization of fluorescent lipid and DNA components, Gene Ther. 8 (11) (2001) 828–836.
[25] S. Li, L. Huang, In vivo gene transfer via intravenous administration of cationic lipid–protamine–DNA (LPD) complexes, Gene Ther. 4 (9) (1997) 891–900.

[26] M. Whitmore, S. Li, L. Huang, LPD lipopolyplex initiates a potent cytokine response and inhibits tumor growth, Gene Ther. 6 (11) (1999) 1867–1875.
[27] F. Sakurai, R. Inoue, Y. Nishino, A. Okuda, O. Matsumoto, T. Taga, F. Yamashita, Y. Takakura, M. Hashida, Effect of DNA/liposome mixing ratio on the physicochemical characteristics, cellular uptake and intracellular trafficking of plasmid DNA/cationic liposome complexes and subsequent gene expression, J. Control. Release 66 (2–3) (2000) 255–269.
[28] G.B. Lipford, K. Heeg, H. Wagner, Bacterial DNA as immune cell activator, Trends Microbiol. 6 (12) (1998) 496–500.
[29] D. Majhen, A. Ambriovic-Ristov, Adenoviral vectors — how to use them in cancer gene therapy? Virus Res. 119 (2) (2006) 121–133.
[30] Q. Liu, D.A. Muruve, Molecular basis of the inflammatory response to adenovirus vectors, Gene Ther. 10 (11) (2003) 935–940.
[31] T.E. Shenk, in: D.M.K., P.M. Howley (Eds.), Adenoviridae: The Viruses and their Replication, Lippincott Williams & Wilkins, Philadelphia, 2001, pp. 2265–2300.
[32] F. Liu, H. Qi, L. Huang, D. Liu, Factors controlling the efficiency of cationic lipid-mediated transfection in vivo via intravenous administration, Gene Ther. 4 (6) (1997) 517–523.
[33] G. Osaka, K. Carey, A. Cuthbertson, P. Godowski, T. Patapoff, A. Ryan, T. Gadek, J. Mordenti, Pharmacokinetics, tissue distribution, and expression efficiency of plasmid [33P]DNA following intravenous administration of DNA/cationic lipid complexes in mice: use of a novel radionuclide approach, J. Pharm. Sci. 85 (6) (1996) 612–618.
[34] L.C. Mounkes, W. Zhong, G. Cipres-Palacin, T.D. Heath, R.J. Debs, Proteoglycans mediate cationic liposome–DNA complex-based gene delivery in vitro and in vivo, J. Biol. Chem. 273 (40) (1998) 26164–26170.
[35] K. Yasuda, H. Kawano, I. Yamane, Y. Ogawa, T. Yoshinaga, M. Nishikawa, Y. Takakura, Restricted cytokine production from mouse peritoneal macrophages in culture in spite of extensive uptake of plasmid DNA, Immunology 111 (3) (2004) 282–290.
[36] K.J. Ishii, S. Akira, Innate immune recognition of, and regulation by, DNA, Trends Immunol. 27 (11) (2006) 525–532.
[37] S. Fletcher, A. Ahmad, E. Perouzel, A. Heron, A.D. Miller, M.R. Jorgensen, In vivo studies of dialkynoyl analogues of DOTAP demonstrate improved gene transfer efficiency of cationic liposomes in mouse lung, J. Med. Chem. 49 (1) (2006) 349–357.
[38] J.S. Zhang, F. Liu, L. Huang, Implications of pharmacokinetic behavior of lipoplex for its inflammatory toxicity, Adv. Drug Deliv. Rev. 57 (5) (2005) 689–698.

アデノウイルスベクター開発の最前線

水口裕之

従来のアデノウイルスベクターの問題点（免疫反応を生じることや感染域の制限など）を克服し，機能性（ターゲティング能の付与など）に優れた次世代アデノウイルスベクターの開発が進んでいる．本稿では，主に遺伝子工学的手法を用いたアデノウイルスベクターの改良研究の最前線について解説する．

はじめに

遺伝子治療の実用化といっそうの進展に向けての最大の鍵は，高い安全性を確保し，治療用目的遺伝子を必要な細胞に効率良く導入し，目的に応じて自由に発現させる技術の開発である．これまで施行されてきた遺伝子治療臨床研究は，主に1990年代に開発された古典的なベクターを用いたものであり，米国でのアデノウイルスベクター投与に伴う死亡事故や，フランスでの白血病の発症といった例外的な事例はあるものの，安全面での評価はほぼ終わり，今後はより有効性に重点を置いた臨床研究が主流になってくるものと思われる．そのためには，機能面で優れた改良型ベクターの開発と実用化が必要不可欠であり，これにより遺伝子治療の安全性・有効性の大幅な増進が期待できる．

アデノウイルスベクターは，①既存のベクターでは最も遺伝子導入効率が良いこと*1，②導入遺伝子が宿主染色体へ組み込まれることなく，染色体外にエピゾームとして存在することから，一過性の遺伝子発現を示すこと*2，③他のウイルスベクターに比べ圧倒的に高いタイター（力価）のベクター（通常他のベクターに比べ1,000倍以上）が得られること，などの長所を有し，ベクターとしての優れた基本的性質を有している．

一方，①遺伝子導入が標的細胞のアデノウイルス受容体（coxsackievirus and adenovirus receptor：CAR）の発現レベルに依存し，CARを発現していない細胞への適用が困難なこと，②組織特異性を示さないこと，③免疫反応を伴うこと，などの問題点を有し，これらの問題を克服し，機能面で優れた次世代アデノウイルスベクターの開発がわれわれや欧米を中心に盛んに行われている．本稿では，次世代アデノウイルスベクター開発の最前線について解説する．

1．標的細胞指向性の制御

i）遺伝子導入時のCAR依存性を克服したアデノウイルスベクターの開発

従来用いられているアデノウイルスベクターは，サブグループCに属する5型（あるいは2型）のヒトアデノウイルスを基盤としている．ヒトアデノウイルスはAからFまでのサブグループに分けられ，少なくとも51種類のserotypeが知られているが，サブグループBに属するウイルスを除き，多くのアデノウイルスは受容体としてCARを認識して細胞に感染する（図1）[1]．CARの発現が乏しいために従来の5型アデノウイルスベクターでの効率の良い遺伝子導入が困難な細胞種は意外と多く，造血幹細胞をはじめとする血液系細胞，樹状細胞，間葉系幹細胞，血管平滑筋細胞，骨格筋細胞，滑膜細胞などが知られている．また，癌細胞は悪性度の進行とともに，CARの発現低下，およびアデノウイルスベクターでの遺伝子導入効率の低下が報告されており[2]，本ベクターを用いて癌を対象とした遺伝子治療臨床研究を進めるうえで考慮すべき問題と考えられている．

＊1：センダイウイルスベクターも遺伝子発現効率に優れていることが報告されているが，本ベクターは他の一般的なウイルスベクターと異なり，細胞内に導入された遺伝子が増幅するという特異な性質を有することから，他のベクターと同列に比較することは困難である．

＊2：細胞増殖に伴い導入遺伝子が希釈されるため，一過性の遺伝子発現を示す．一方，分化した増殖停止期の細胞に対しては，後述するようにアデノウイルスに対する免疫の問題が克服されれば，数ヶ月以上の長期間の遺伝子発現を示すことが知られている．

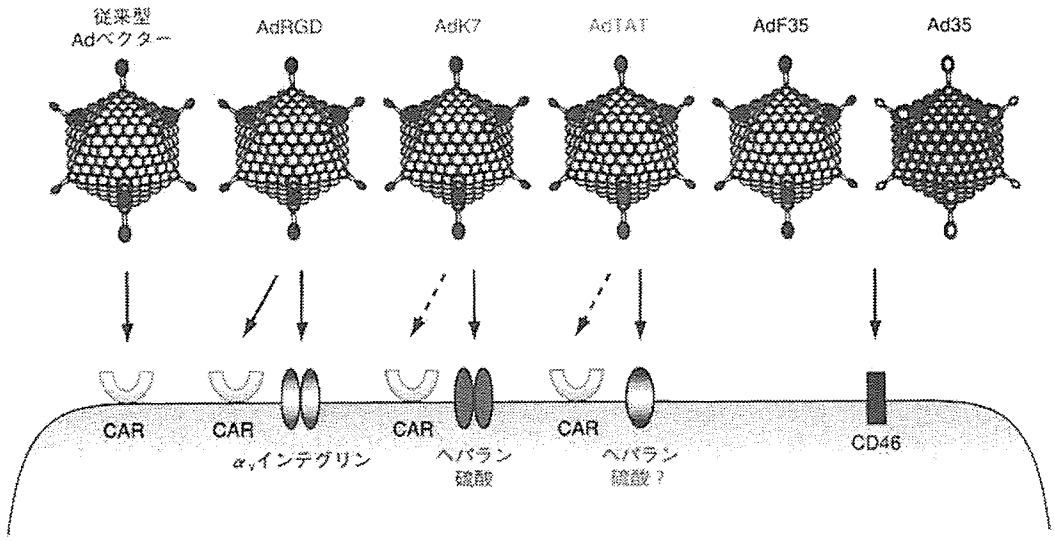


図1 ●各種改良型アデノウイルスベクター

野生型のファイバーをもった従来型の5型アデノウイルスベクターは細胞表面上の受容体であるCARを認識して感染するが，RGD配列やポリリジン配列をファイバーに有したファイバー改変ベクター（AdRGD，AdK7）はCARだけでなくαvインテグリンやヘパラン硫酸を認識しても感染できる．TATペプチドを付与したAdTATは，詳細な細胞内移行メカニズムは不明であるが，CAR非依存的に感染できる．また，35型アデノウイルスのファイバーを有したベクター（AdF35）や，すべての構造タンパク質が35型アデノウイルスからなるベクター（Ad35）は，CD46を認識して感染する

アデノウイルスベクターによる遺伝子導入時のCAR依存性を克服するために，ファイバータンパク質[*3]を改変した改良型ベクターの開発が進んでいる．例えば，αvインテグリンに親和性があるRGD（Arg-Gly-Asp）ペプチドや，ヘパラン硫酸に親和性があるポリリジンペプチドをファイバー表面上に遺伝子工学的に表現させることにより，CARを発現していない細胞に対しても効率良く遺伝子導入できる（図1）[3,4]．われわれは最近，HIV（human immunodeficiency virus）由来のタンパク質導入ドメイン（Protein Transduction Domain：PTD）として知られているTatペプチドをファイバーに付与することで，RGD配列やポリリジン配列を付与したベクターよりも，より広範に効率良く外来遺伝子を発現可能であることを見出し，その応用が期待される．

また，ファイバー部位をCAR以外の分子を認識する他の血清型のアデノウイルスのファイバーに置換することでも，遺伝子導入時のCAR依存性を克服することができる．例えば，ヒト由来細胞であればほとんどすべての細胞に発現が認められるCD46を受容体としている11・35型アデノウイルス由来のファイバーを付与することで，5型アデノウイルスベクターでの効率の良い遺伝子導入が困難な造血幹細胞，樹状細胞などへの効率の良い遺伝子導入が可能になる[5,6]．

ii）ターゲティング能を有した
アデノウイルスベクターの開発

ターゲティング能を有したベクターの開発は，全身投与での治療効果が期待できるだけでなく，たとえ局所にベクターを投与した場合においても，標的細胞以外への感染，拡散を防ぐことが期待できることから，重要な研究課題である．目的の組織でだけ遺伝子を発現させることが可能なアデノウイルスベクターの開発には，①キャプシドタンパク質の遺伝子工学的な改変，②抗体やタンパク質，高分子を用いてのベクター表面の修飾，あるいは③組織特異的プロモーターの利用などの方法がある．最終的には，これらの組み合わせが好ましいが，ベクター自身を

＊3：ファイバータンパク質
テール，シャフト，ノブからなり，ノブ領域がCARと結合する．

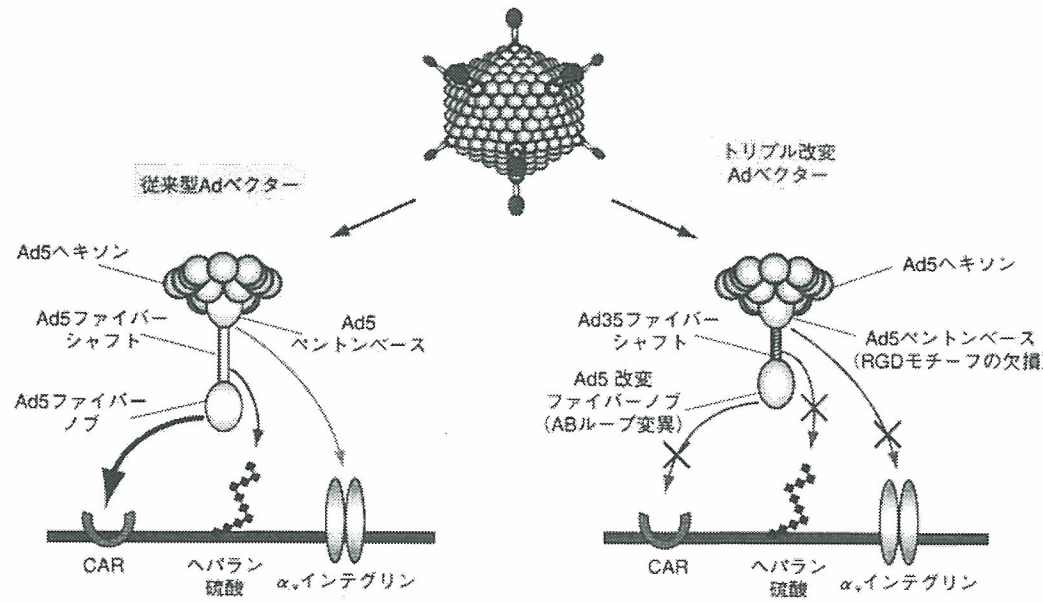


図2●ターゲティング能を有したアデノウイルスベクター

ターゲティング能を有したアデノウイルスベクターの開発のためには，まず，①ファイバーノブとCARとの結合を介した感染ルートを回避し，②低親和性であるがペントンベースのRGDモチーフがα_vインテグリンと直接結合することによって起こる感染ルートを回避し，③ファイバーシャフト領域がヘパラン硫酸に作用することによって起こる感染ルートも回避したベクター（トリプル改変ベクター）を開発する必要がある．次に，トリプル改変ベクターに標的細胞特異的に結合するリガンドなどを付与することで，ターゲティング能を有したアデノウイルスベクターが開発できる

改変する①が最も基本的で重要な基盤技術になると考えられ，精力的に研究が進められている．

アデノウイルスは前述のようにファイバーとCARとの結合が感染に重要な役割を果たし，低親和性であるがファイバーの根本に存在するペントンベースのRGDモチーフやファイバーシャフトのKKTKからなるヘパリン結合ドメインが，α_vインテグリンやヘパラン硫酸と結合して起こる感染ルートも知られている[7]．また最近では，factor Xなどの血液成分がアデノウイルスと細胞との結合を橋渡しして，受容体非依存的に感染するルートも報告されている[8]．これらの感染経路を遮断して，標的細胞特異的に結合するリガンドなどをウイルス表面タンパク質のファイバーやヘキソン，protein IX領域*4（われわれはこれらの領域に簡便に外来リガンドを挿入する技術も開発済みである[9]）に付与すれば，ターゲティング能をもったアデノウイルスベクターが開発できる．われわれはファイバーノブ，シャフト，ペントンベースの3領域を同時に改変したトリプル改変ベクターを開発し（図2），このベクターが肝臓をはじめとする*in vivo*での遺伝子発現能をほとんど消失していることを明らかにしている[10][11]．現在このトリプル改変ベクターに，標的細胞特異的に高親和性を示すリガンドを付与することでターゲティング能をもったアデノウイルスベクターの開発を進めている．

2. 免疫反応の制御

アデノウイルスベクターを生体に投与した場合，大別すると，①ベクター投与後数時間以内に生じるベクター粒子そのものに対する自然免疫，②7～10日以内に生じるベクターにより産生されたウイルスタンパク質（および外来遺伝子産物）に対する細胞性免疫，そして，③ウイルスタンパク質に対する液性免疫の3種類の免

＊4：protein IX領域
ヘキソンとヘキソンの間に存在するタンパク質．

疫反応が起こることが報告されている．これらの免疫反応を克服できるベクターや投与方法（投与戦略）の開発は，安全性や有効性の高い遺伝子治療の実現にとって必要不可欠である．

i）自然免疫の制御が可能な
アデノウイルスベクターの開発

ウイルスをはじめとする異物を生体に投与すると，生体は即座にインターフェロンやサイトカイン，ケモカインなどを産生し，それらの異物を排除しようとする自然免疫が発動する．アデノウイルスベクターを生体に投与した場合にも自然免疫の活性化は生じ，1999年に米国で起こったオルニチントランスカルバミラーゼ欠損症に対するアデノウイルスベクターを用いた死亡事故では，自然免疫の過剰な活性化が原因と考えられている．

アデノウイルスベクターをマウスに全身投与した場合には，IL-6，IL-12などの産生が投与3時間以内に生じる．全身投与されたベクターの90％以上は肝臓（実質細胞とクッパー細胞*5をはじめとする非実質細胞）に移行するが，多くの炎症性サイトカインは肝臓（のクッパー細胞）ではなく，主に脾臓で分泌される[10]．したがって，脾臓への移行性を抑えたベクターは，炎症性サイトカインの産生が低い．例えば，前述したポリリジンでファイバーを修飾したアデノウイルスベクターでは，脾臓への移行性が減少する結果，血中へ分泌されるIL-6は約1/4にまで減少する（肝臓をはじめとする各臓器での遺伝子発現は従来型ベクターと同等以上に起こる）[10]．また，CAR，インテグリン，ヘパラン硫酸との結合性を欠損させたトリプル改変ベクターにおいては，IL-6はほとんど産生されない[11]．興味深いことに，従来のアデノウイルスベクターを投与した場合に生じるAST（aspartate aminotransferase）やALT（alanine transferase）などの肝傷害性マーカーの産生も，ポリリジン型ベクターやトリプル改変ベクターではほとんど全く起こらず[10][12]，安全性が高いことが明らかとなっている．

ウイルスなどの病原体を認識する細胞上のセンター受容体として，近年TLR（Toll Like Receptor）が注目されている．TLR3を除くTLRが利用しているアダプタータンパク質のMyD88欠損マウスを用いたわれわれの検討では，アデノウイルスベクター刺激による樹状細胞でのIL-6産生にはMyD88が関与するものの，マクロファージでのIL-6産生にはMyD88には関与せず，細胞種によりIL-6の産生メカニズムが異なっていることが明らかとなった[13]．さらに*in vivo*での血中IL-6の産生は，MyD88欠損マウスにおいても野生型マウスと同程度に起こり，TLR以外の経路が*in vivo*におけるIL-6産生に大きく寄与していることが示唆された．アデノウイルスベクター投与に伴う自然免疫に関与する生体側因子やウイルス側因子，シグナル伝達機構の解明は，自然免疫応答の克服に向けて重要な研究課題であり，われわれのグループでは，各種改変アデノウイルスベクターやノックアウトマウス，RNAi技術，マイクロアレイ解析を通して，これらの研究を進めている．

ii）細胞性免疫の制御が可能な
アデノウイルスベクターの開発

アデノウイルスベクターを生体に投与すると，通常数週間から数カ月間の一過性の遺伝子発現を示す．しかしながら，免疫不全マウスにおいては，数カ月から1年以上（場合によっては一生涯）にわたる長期間の遺伝子発現を示すことから*6，免疫系による遺伝子導入細胞の排除が一過性の遺伝子発現の原因と考えられている．すなわち，従来のアデノウイルスベクターは，ウイルスの増殖やウイルスタンパク質の合成に必須のE1遺伝子領域を除去することで，ウイルスタンパク質の産生が生じないように設計されているが，E1遺伝子非依存的に他のウイルスタンパク質の合成がわずかながら起こり，これが免疫系のターゲットとなることが明らかになっている．この問題を克服するために，ウイルスコード遺伝子をすべて除去したgutted アデノウイルスベクターが開発されており，本ベクターを

＊5：実質細胞とクッパー細胞
肝臓は肝実質細胞（肝細胞）の他に，クッパー細胞，類洞内皮細胞，星細胞，胆管上皮細胞などの肝非実質細胞から構成される．

＊6：
増殖停止期の終末分化した細胞に遺伝子導入した場合には，導入遺伝子が染色体外にエピゾームとして存在するアデノウイルスベクターにおいても長期間の遺伝子発現を示す．

用いた場合は，通常のマウスにおいても長期間の遺伝子発現が認められる[18]．従来は，高タイターのgutted アデノウイルスベクターの産生が技術的に難しいのが課題点であったが，最近はヘルパーウイルスとパッケージング細胞の改良により，その問題点は一部克服されつつある[35]．また，gutted アデノウイルスベクターに，Sleeping Beauty[36] やバクテリオファージインテグラーゼφC31[37]，レトロトランスポゾン[38] の染色体への遺伝子組み込み活性を付与することで，導入遺伝子が積極的に染色体に組み込まれる性質をもたせたアデノウイルスベクターの開発も進められており，このようなベクターでは分裂細胞においても永続的な遺伝子発現が期待できる．

iii）液性免疫の制御が可能な
アデノウイルスベクターの開発

成人の約60％はヒト5型アデノウイルス（サブグループCに属する）に対する抗体を保持していることが知られている．また，アデノウイルスベクターを投与された個体は，抗アデノウイルス抗体を生じるため，2回目以降のベクターの全身投与では遺伝子発現をほとんど示さない*7．主要キャプシドタンパク質のヘキソンに対する抗体が液性免疫の主体であることから，ヘキソン改変ベクターが，この問題を克服するために開発されている[19]．また，異なった血清型（サブグループBに属する11型や35型）や異なった種（チンパンジー，イヌ，ヒツジ，トリ，ウシ，マウスなど）に属するアデノウイルスベクターが開発されており，これらのなかには，CAR以外の受容体を認識して感染するものもあり，感染域を変えることも可能になる．例えば，われわれはすべての構造タンパク質が35型アデノウイルスからなるベクターの開発に成功したが[21]，35型アデノウイルスに対する成人の抗体保持率は低く，本ベクターは5型アデノウイルス抗体存在下でも高い遺伝子発現を示す．35型アデノウイルスはCD46を受容体として感染し，造血幹細胞などの5型アデノウイルスベクターでの遺伝子導入が困難な細胞への遺伝子導入に優れていることが明らかとなっている[3,7,20]．

3．基礎研究への応用

アデノウイルスベクターはわれわれが開発したプラスミド構築に基づいた簡便なベクター作製法が開発されたこと[21,22]（クロンテック社よりキット化），高力価のウイルス液が得られること，遺伝子発現効率が高いことなどから，遺伝子機能解析などの基礎研究のための貴重なツールとして広く利用されている．さらに，前述した改良型ベクターにより，従来のベクターでは遺伝子導入が困難であった多くの細胞種への劇的な遺伝子導入効率の改善が可能になっており，汎用性は益々高くなっている．

アデノウイルスベクターは全身投与した場合には，肝臓に90％以上のベクターが移行し，100％の肝細胞で遺伝子発現が起こる．また，臓器局所にベクターを投与した場合には，投与部位での高効率な遺伝子発現が期待できる．目的組織がCAR陰性の場合は，ファイバーを改変したベクターを用いることで遺伝子発現効率の上昇が期待できる．例えば，CAR陰性のB16メラノーマの腫瘍内にベクターを投与した場合には，RGDペプチドを付与したファイバー改変ベクターでは従来型ベクターに比べ約40倍もの遺伝子発現を示す[23]．また，遺伝子導入が物理的な問題により容易でない膵臓ランゲルハンス島（膵島）β細胞に対しては，腹腔動脈からアデノウイルスベクターを *in vivo* 投与し，その後膵島を初代培養することで高効率な遺伝子発現が得られることが明らかとなっており（ランゲルハンス島の内部の細胞においても，高効率な遺伝子発現が得られる）[24]，糖尿病の分子メカニズムの解明や治療に向けた研究分野への強力なツールになると期待される．さらに，35型アデノウイルスの受容体であるCD46を全身の臓器で発現しているトランスジェニックマウス（CD46は齧歯類ではほとんど発現していない）を用いた各組織局所への35型ベクターを用いた遺伝子導入系は，基礎研究における実験系としてきわめて有効で

*7：局所投与された場合は，複数回の投与でも有効である．

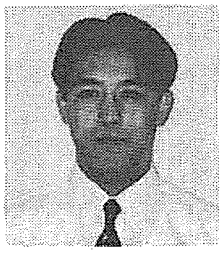
ある[25]．

おわりに

本稿で紹介したように，アデノウイルスベクターの改良はさまざまな方向から精力的に進められている．最終的には，前述の各種改変技術を組み合わせたり，化学的手法によるベクター改変や組織特異的プロモーターの利用，導入する個々の遺伝子（あるいはsiRNA発現ベクターとして）の特性を考慮に入れて活用することで，アデノウイルスベクターは遺伝子治療への応用や基礎研究のツールとして益々有効な技術になると考えられる．今後，このような改良型アデノウイルスベクターの開発が，遺伝子治療臨床研究の成功や遺伝子治療の普及，さらには生命科学研究の発展という成果となって現れることを期待している．

参考文献

1）Bergelson, J. M. et al.：Science, 275：1320-1323, 1997
2）Okegawa, T. et al.：Cancer Res., 61：6592-6600, 2001
3）Mizuguchi, H. et al.：Gene Ther., 8：730-735, 2001
4）Koizumi, N. et al.：J. Gene Med., 5：267-276, 2003
5）Sakurai, F. et al.：Gene Ther., 10：1041-1048, 2003
6）Rea, D. et al.：J. Immunol., 166：5236-5244, 2001
7）Mizuguchi, H. et al.：Hum. Gene Ther., 15：1022-1033, 2004
8）Shayakhmetov, D. M. et al.：J. Virol., 79：7478-7491, 2005
9）Kurachi, S. et al.：Gene Ther., 14：266-274, 2007
10）Koizumi, N. et al.：J. Virol., 77：13062-13072, 2003
11）Koizumi, N. et al.：Hum. Gene Ther., 17：264-279, 2006
12）Koizumi, N. et al.：J. Immunol., 178：1767-1773, 2007
13）Yamaguchi, T. et al.：submitted
14）Donna, J. P. et al.：Hum. Gene Ther., 16：1-16, 2005
15）Palmer, D. et al.：Mol. Ther., 8：846-852, 2003
16）Yant, S. R. et al.：Nature Biotechnol., 20：999-1005, 2002
17）Ehrhardt, A. et al.：Mol. Ther., 15：146-156, 2007
18）Kubo, S. et al.：Proc. Natl. Acad. Sci. USA, 103：8036-8041, 2006
19）Roberts, D. M. et al.：Nature, 441：239-243, 2006
20）Sakurai, F. et al.：Gene Ther., 12：1424-1433, 2005
21）Mizuguchi, H. et al.：Hum. Gene Ther., 9：2577-2583, 1998
22）Mizuguchi, H. et al.：Hum. Gene Ther., 10：2013-2017, 1999
23）Mizuguchi, H. et al.：Cancer Gene Ther., 9：236-242, 2002
24）Mukai, E. et al.：J. Cont. Rel., in press
25）Sakurai, F. et al.：Gene Ther., 13：1118-1126, 2006



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