Development of antigen presenting cell specific nucleic acid carrier using β-1,3-glucan

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ABSTRACT SUMMARY
To silence the target gene for several diseases, antisense and RNA interference technology is expected as a next generation therapy. However, lack of a suitable delivering carrier is the major obstacle to practical usage. In this study, we present a novel complex consisting of β-1,3-glucan and nucleic acid. We used a β-1,3-glucan schizophyllan (SPG) and antisense or short interference RNA (siRNA). The complex showed higher affinity to recombinant dectin-1 than SPG itself, where dectin-1 is a β-1,3-glucan receptor expressed on antigen presenting cells and can be a target for specific delivery. The complex suppressed lipopolysaccharide (LPS)-induced TNF-α secretion by peritoneal macrophages in vitro. The complex significantly decreased the serum TNF-α level for the mouse model of LPS-induced acute hepatitis. This new nucleic delivery system may overcome the problem for nucleic acid therapy because of its non-toxicity and high target specificity.

INTRODUCTION
Antisense oligonucleotides (AS-ODNs) or short interference RNAs (siRNAs) have proved potentially efficacious in the treatment of various diseases caused by disorders of gene expression. However, the lack of an appropriate delivery tool to protect nucleic acids from enzymatic degradation and non-specific adsorption remains the major obstacle to practical use.

Some members of a family of natural polysaccharides called β-1,3-glucans are approved for clinical use in Japan and have been used for the treatment of uterine cancer [1]. It has been demonstrated that β-1,3-glucans can form novel complexes with homo ODNs via a combination of hydrogen bonding and hydrophobic interactions [2, 3]. β-1,3-glucans adopts triple helix in neutral water and the helix dissociates into single chains in dimethylsulfoxide or alkaline solution (> 0.25 N NaOHaq). When the solvent of the single chains is substituted with neutral water, the original triple helix is regenerated. When a particular polynucleotide such as poly(C) or poly(dA) is present in this process, two main-chain glucoses of β-1,3-glucans and one ODN base form a stoichiometric complex instead of retrieving the original triple helix. Among β-1,3-glucans, we have focused on schizophyllan (SPG; Figure. 1), because medical-grade SPG is available and its molecular characters are well understood. Another advantage to use β-1,3-glucans is that the complex is presumably recognized by dectin-1 on antigen-presenting cells (APCs) and then taken up. Dectin-1 is a major receptor involved in the recognition of β-glucans on APCs, including macrophages, dendritic cells, monocytes, neutrophils, and a subset of T cells [4, 5]. Therefore, the complex could be used to deliver a nucleic acids that can rectify abnormalities associated with inflammation.

EXPERIMENTAL METHODS
Preparation of S-oligo complex with SPG
SPG was dissolved in 0.25 N NaOHaq for 2 - 5 days to dissociate triple helix to single chain. The SPG solution, AS-ODN or siRNA in water, and phosphate buffer solution (330 mM NaH2PO4, pH = 4.7) were mixed. After mixing, the mixture (nucleic acid 60 μM, pH = 7.4) was stored at 4도C overnight. The molar ratio ([SPG] / [nucleic acid]) was controlled to 0.27.

Gene silencing in vitro
Thioglycolate-elicited peritoneal macrophages (thio-Mφs) were plated at 1.0 × 10⁵ cells/well in 96-well plates, and incubated
for 24 hours. The cells were added antisense/SPG or siRNA/SPG at indicated concentrations and incubated for 2 hours at 37 °C. After the additional incubation for 24 hours in a fresh medium, the cells were stimulated with LPS (10 ng/ml) for 5 hours. The amounts of TNF-α released into the supernatants were measured with ELISA.

Gene silencing for acute hepatitis model

C57BL/6 mice weighing 16-21 g (7-8 weeks old) were treated i.p. with AS-ODN/SPG or siRNA/SPG (PBS was used as a negative control), 24 h later they were co-injected (i.p.) with 600 mg/kg of D-galactosamine (D-GalN) and 10 μg/kg of LPS and AS-ODN/SPG or siRNA/SPG (n = 5). After 1 hour, the blood was obtained by retro-orbital bleeding and the serum TNF-α level was measured with ELISA.

RESULTS AND DISCUSSION

Confirmation of nucleic acid/SPG complex

We confirmed the complexation with field flow fractionation by monitoring UV absorbance at 260 nm before and after complexation of AS-ODN with the (dA)60 tail. After the complexation, there was only one peak, although it was broadened and shifted to a later elution time. This indicates that AS-ODN was indeed complexed with SPG at almost 100%. This was confirmed by PAGE.

Gene silencing in vitro

The 30 nM and 10 nM of siRNA doses suppressed TNF-α expression by 60-70%, while the corresponding naked dose did not. No significant suppression was observed when the dose was decreased to 1 or 3 nM. For the same in-vitro assay, we had to increase the TNF-α antisense dose up to 500 nM to attain the same suppression. We achieved the efficient gene suppression at significant low dose.

Gene silencing for acute hepatitis model

The treatment with 0.1 mg/kg of the siRNA/SPG complex significantly reduced TNF-α, while a naked treatment did not cause any reduction (Figure 2). When we treated same model using AS-ODN/SPG, we needed to increase the dose up to 5 mg/kg to achieve the sufficient suppression. In this study, we were able to achieve the same effect with the dose less than one-tenth of that for AS-ODN.

Figure 2. Treatment with the AS-ODN/SPG and siRNA/SPG complex protects mice from fulminant hepatitis.

CONCLUSION This report demonstrates four findings: (1) nucleic acids attaching dA40 and SPG form a novel complex. (2) The complex showed higher affinity toward dectin-1 than SPG. (3) The complex significantly decreased the serum TNF-α level at an extremely low dose for the mouse model of LPS-induced acute hepatitis. These results show that this new delivery system may overcome the problem for nucleic acid therapy because of its non-toxicity and high target specificity.

REFERENCES