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Designing an immunocyte-targeting delivery system by use of beta-glucan

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ABSTRACT

A β-1,3-D-glucan called Schizophyllan (SPG) can form a novel complex with homo oligodeoxynucleotides (ODNs) via the combination of hydrogen bonding and hydrophobic interactions. Dectin-1 is a major receptor involved in the recognition of β-1,3-D-glucans and expressed on antigen presenting cells (APCs) including macrophages, dendritic cells, monocytes, neutrophils, and a subset of T cells. Therefore, the SPG/ODN complex can be used as APCs cell-specific delivery of functional ODNs including unmethylated CpG sequences (CpG-ODNs). In fact, CpG-ODN/SPG complex induced high antibody titers when it was administered to cynomolgus monkeys as adjutant of influenza vaccine. These results indicate that SPG can be an excellent immunocyte-targeting drug delivery system.

1. Introduction

Drug delivery system (DDS) refers to the group of technologies for transporting pharmaceutical compounds to desired organs or cells, avoiding clearance due to kidneys or renal dialysis or degradation due to interactions with blood proteins before reaching its target. Development of good DDS is expected to enable targeting delivery of drugs, resulting in enhancement of clinical effect. We have focused on gene medicine consisting of therapeutic DNA or RNA, which are known as a new medicine in the next generation. The therapeutic ODNs are expected to enable effective medical treatment without side effects because they directly act on gene expression.

Chinese herbal medicine has a long history, probably extending back to about 2500–3000 years ago. In this history, some fungi has been recognized as a potential medicine to cure gynecological diseases [1]. Modern chemistry has then revealed that the active ingredient of the fungi is a polysaccharide belonging to β-1,3-D-glucan family [2]. Schizophyllan (SPG, see Fig. 1 for the chemical structure) [3,4], one of β-1,3-D-glucans family, can form complex with nucleic acids such as poly(C), poly(A), poly(U), poly(dA), and poly(T), and is recognized by antigen presenting cells (APCs). In this report, we introduce physical properties of the complex and the nucleic acid medicine delivery by use of this complex.

2. Application of the complex to functional oligonucleotide carrier

2.1. Recognition of ODN/SPG by the β-1,3-D-glucan receptor; Dectin-1

We demonstrated that therapeutic ODNs, such as unmethylated CpG (CpG-ODNs), coupled with dA (or S-dA) were complexed with SPG and delivered to immunocytes, especially antigen presenting cells (APCs) such as dendritic cells and macrophages. Gordon et al. found a major β-1,3-D-glucan receptor called Dectin-1, expressed on the surface of APCs such as murine peritoneal macrophages and dendric cells [5]. We presume that then therapeutic ODN/SPG complex can be recognized by Dectin-1 and induce the uptake of the bound therapeutic ODNs without chemical modification such as ligand. If this hypothesis is correct, it can be possible to specifically deliver therapeutic ODNs into the APCs. In this section, we introduce SPG having specificity for APCs as DDS carrier.

2.2. IL-12 production due to administration of the CpG-ODN/SPG complex

The innate immune system has the remarkable ability to distinguish a subtle difference between pathogenic and host molecules with the aid of TLRs [6,7]. In particular, toll-like receptor (TLR9) is located in the endocytosis compartments of APCs, and lowering the pH at the late endocytosis can lead TLR9 to bind ODN having unmethylated CpG sequences (CpG-ODNs) [8,9]. A recent study has shown that dimerization of TLR9 is critical to its activation by CpG-ODN [10]. After the activation, NF-κB is recruited through a MyD88-dependent pathway, and eventually, a Th1 response is

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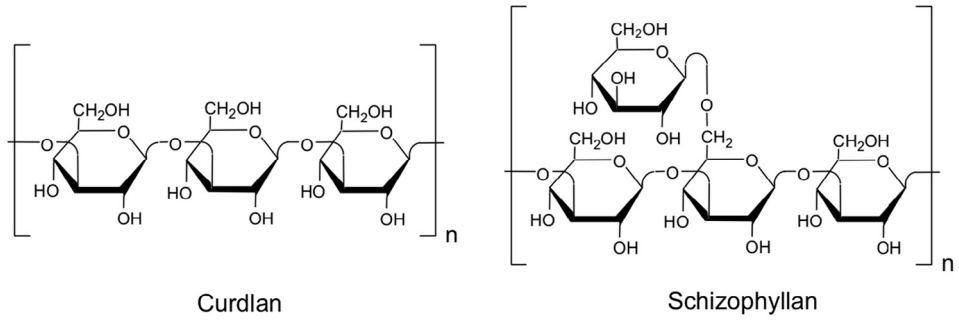

Fig. 1. Chemical structures of curdlan and schizophyllan.

initiated, following the production of various cytokines including IL-12. The optimal CpG motifs for murine TLR9 are defined as an unmethylated CpG dinucleotide flanked by two 5′ purines and two 3′ pyrimidines, i.e., Pu-Pu-C-G-Py-Py [11]. Methylation of the cytosine or sequence inversion from CpG to GpC does not activate TLR9 at all, indicating that this recognition is highly sensitive to molecular structure [12]. In the past 10 years, the molecular mechanism of CpG-ODN/TLR9 interactions has gradually become understood [13]. In accordance with this, clinical trials of CpG-ODN have been conducted for allergies, asthma, cancers, and certain infectious diseases [14]. In this section, we introduce DDS for CpG-ODN as one of the immune adjuvant.

Fig. 2(a) compares polyacrylamide gel electrophoresis patterns between CpG-dA_{60} (TCC ATG ACG TTC CTG ATG CT-dA_{60}) and its mixture with SPG. With increase of [mG]/[dA], the naked CpG-dA_{60} band faded away. Here [mG] and [dA] mean the molar concentration of the main-chain glucose of SPG and the dA base of CpG-dA_{60}, respectively. The ratio of complexation deter-mined by fluorescence contrast was 56% in lane 2, 78% in lane 3, 96% in lane 4, and 100% in lane 5–8, respectively.

Peritoneal macrophages express TLR9 and induce IL-12 by stimulation with CpG-ODN [15]. We added the complexes with various compositions to the peritoneal macrophages [16], and measured the amount of secreted IL-12 with ELISA (Fig. 2(b)). In the range of [mG]/[dA] = 0–1.0, the production slightly increased with increase of the amount of SPG, and dramatically enhanced at [mG]/[dA] = 2. At [mG]/[dA] = 5, the complex induced about 7-fold higher production than that of naked CpG-dA_{60}, and the production decreased with further increase of amount of SPG. Interestingly, between [mG]/[dA] = 1 and 5, there is a significant difference in the IL-12 production from cells, while the amount of CpG-dA_{60} taken up by the cells differed only slightly among them [17].

We have showed that the complex is more flexible than SPG. Our recent study has shown that the flexibility of the polymer containing CpG-ODN is a key to induce the large amount of IL-12 [18]. Once one CpG branch binds to one TLR9 dimer, the SPG is locked adjacently to the vesicle surface where other TLR9 dimers are present. When the structure is flexible enough to turn, the second binding can easily occur, and the adjacent second binding seems to be critical for the allosteric effect. At [mG]/[dA] < 2 in Fig. 2(b), the complex is considered to have the maximum number of CpG-dA_{60} due to the CpG-dA_{60}-rich composition. On the other hand, at [mG]/[dA] > 2, most of the complexes have fewer CpG-dA_{60} than that at [mG]/[dA] < 2 due to the SPG-rich composition. With increasing [mG]/[dA], the flexibility of the complex is considered to increase. The complex with rigid structure could be difficult to access the adjacent TLR9 dimers, while the complex with flexible structure could access them easily. Therefore, the cytokine secretion drastically increased at [mG]/[dA] > 2 because of the easy accessibility of the complex to TLR9 dimers. These results suggested the existence of a particular higher-order structure to activate TLR9 more efficiently. We found that SPG is highly effective even in the delivery of adjuvant. In the next section, we introduce therapeutic effect by CpG-ODN/SPG complex against mice and cynomolgus monkeys.

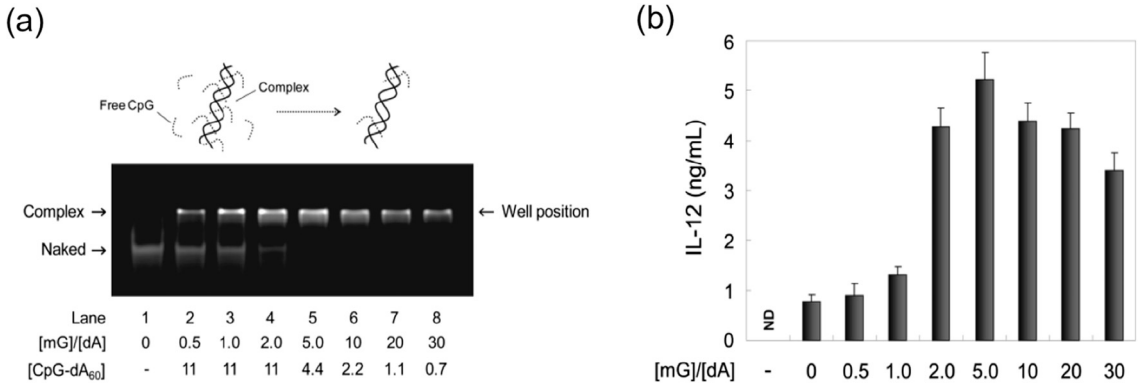

Fig. 2. Complexation of CpG-dA_{60} and SPG with SYBR Gold stain. [CpG-dA_{60}] indicates the number of CpG-dA_{60} molecules included in one complex that is calculated from [mG]/[dA]. The upper illustrations show how the SPG and CpG-dA_{60} molecules exist in the solutions; at [mG]/[dA] < 2, there is free CpG-dA_{60} and the complex is fully loaded with CpG-dA_{60}, while at [mG]/[dA] > 2, there is uncomplexed SPG and the complex has fewer CpG-dA_{60} (a). The complex composition dependence (CpG-dA_{60}: 0.5 μM) of IL-12 production from the murine peritoneal macrophages after incubation for 24 h. The abbreviation (ND) means "not detectable" (b) (Permission obtained from American Chemical Society).

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