CpG delivery using antigen-presenting cell specific carrier polysaccharide for immunotherapy

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ABSTRACT SUMMARY
CpG known as an adjuvant has been treated for allergies, asthma, cancer, and certain infectious disease. We have studied an interaction between a polysaccharide schizophyllan (SPG) and nucleic acid with CpG. SPG is recognized receptor for β-1,3-glucan on the antigen-presenting cell. We made crosslinking complex through DNA hybridization. From gel electrophoresis analysis, the size of crosslink showed larger than of conventional complex. Treatment with crosslink induced the highest cytokine production. These results indicate that crosslink of complexes can be a candidate for new adjuvant delivery.

INTRODUCTION
A number of diseases with compromised immune system don’t have effective therapy. CpG-DNA is expected as a novel adjuvant since the antigen-presenting cell of mammal is known to activate a Th1 immune response by recognizing a non-methyl CpG. Toll like receptor 9 (TLR9) exists in endosome and recognizes a non-methyl CpG (Figure 1). Therefore the activation of cell using a CpG needs drug delivery system.

β-1,3-glucan is one of the polysaccharide and is abundant on earth. Here we used schizophyllan commune Fries as extracellular polysaccharide. It consists of the main chain β-1,3-glucan and one β-1,6-glycosyl side chain that links to the main chain of every three glucose residues (Figure 2.a).

We have studied SPG-nucleic acid interaction. After being dissolved in alkaline solution (>0.25 N NaOHaq), a triple helix of SPG dissociates and become the random coiled single chain. (1) The triple helix is retrieved when the solvent was neutralized in a neutral solution. We found that the new complex which one of triple helix chains was replaced by a nucleic acid nucleic acid is formed when poly(dA) and poly(C) existed in this process (Figure 2.b),(2,3)

SPG is recognized by β-1,3-glucan receptor on the antigen-presenting cells (Figure2.c). In this study, we made cross-linking complex through DNA hybridization between complexes and evaluated the ability to activate immune cells.

EXPERIMENTAL METHODS

- Design of carrier

We used two kinds of nucleic acids for complexation. One is CpG with dA40, and the other is complementary CpG (cCpG) with dA40. Crosslink complex is formed by mixing CpG/SPG and cCpG/SPG complex. (Figure 3)

Figure 1. The CpG is activated the antigen-presenting cell

Figure 2. a) The structure of SPG b) Complex made from SPG and DNA c) Dectin-1 recognizes β-glucan
Figure 3. Design of crosslink by DNA/SPG complexes

- Cytokine IL-6 production
  Each size of CpG, complex and crosslink complex is evaluated with poly acrylamide and agarose gel electrophoresis. After incubating peritoneal macrophages overnight, we evaluated the cell activation by CpG, complex, crosslink complex with enzyme-linked immune sorbent assay (ELISA) for IL-6.

RESULTS AND DISCUSSION

- Gel electrophoresis
  The bands for complex and crosslink complex were shown at higher molecular weight position than nucleic acid by PAGE. The band for crosslink complex was shown at higher position than complex.

- IL-6 production
  The cell recognizes non-methylated CpG and induces IL-6 (Figure 4.a). The treatment with complex induced higher IL-6 production than that with CpG (Figure 4.b). Crosslink complex induced IL-6 more than CpG-DNA/SPG complex (Figure 4.c).

CONCLUSION

Crosslink complex can make from complementary complex. Complex was most produce cytokine IL-6. Crosslink is expected as a carrier of adjuvant with high ability to be recognized than complex.

REFERENCES