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Enhancement of immunostimulatory effect of CpG oligodeoxynucleotide by
using boron nitride nanospheres
(窒化ホウ素ナノ粒子を用いた CpG オリゴデオキシヌクレオチドの免疫活性化能の向上)

Bacterial and viral DNA containing unmethylated cytosine-phosphate-guanine (CpG) dinucleotides stimulate the mammalian innate immune system. This process is mediated by the activation of Toll-like receptor 9 (TLR9), a member of Toll-like receptor family. Synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs are like those found in bacterial DNA and possess similar immunostimulatory effects. The activation of TLR9 initiates an immunostimulatory cascade that induce the maturation, differentiation, and proliferation of multiple immune cells including B and T lymphocytes, natural killer (NK) cells, and monocytes/macrophage. This further triggers cell signaling pathways including mitogen activated protein kinases (MAPKs) and NF κ B, subsequently results in the induction of multiple proinflammatory cytokines and chemokines that modulating the cellular inflammatory response. As such, CpG ODNs have potential for treatment of infectious diseases, allergies, and cancers. However, the immunostimulatory effects are often limited by the poor stability and cellular uptake of natural CpG ODNs. Therefore, there has been great interest in developing approaches to optimize the stimulatory activity of CpG ODNs. Chemical modification of CpG ODNs backbone is an effective technique to protect against degradation by nucleases. However, there is concern over several severe side effects. Since CpG ODNs are negatively charged, it is difficult for them to bind to the negatively charged cell surface. This electrostatic repulsion is believed to limit the efficiency of CpG ODNs uptake and their immunostimulatory effect. Evidence is accumulating indicates that both the stability and cellular uptake of natural CpG ODNs can be enhanced by using nanoparticles as carriers. Therefore, delivery of unmodified CpG ODNs using nanoparticles maybe an good approach to improve their immunostimulatory effect, and make it possible to use naturally occurring CpG ODNs in clinical applications. In this regard, we develop novel delivery systems for CpG ODNs based on boron nitride nanospheres (BNNS) for enhancing the immunostimulatory effect of CpG ODNs.

In chapter 1, an general introduction of this study was given, including the mechanism of human immune system, the interaction between TLR9 and CpG ODNs, therapeutic potential of CpG ODNs, delivery systems for CpG ODNs, and the BNNS.

In chapter 2, a novel CpG ODNs delivery system based on a BNNS-binding peptide has been developed. Firstly, a 12-amino acid peptide, designated as BP7, which had specific affinity for BNNS, was indentified using phage display technique. BNNS that bound BP7 (BNNS/BP7) were taken up into cells and showed no cytotoxicity. Using BP7 as a linker, the loading capacity of CpG ODNs on BNNS increased 5-fold compared to the direct binding of CpG ODNs to BNNS. Then we used the BP7-CpG

ODNs conjugates-loaded BNNS to stimulate the peripheral blood mononuclear cells (PBMCs) and measured the cytokine productions. BP7-CpG ODNs conjugates-loaded BNNS had a greater capacity to induce interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) production from PBMCs than that of CpG ODNs directly loaded on BNNS. However, it could not induce interferon- α (IFN- α) from PBMCs. The higher amount of cytokine induction from BP7-CpG ODNs conjugates-loaded BNNS may be attributed to a higher loading capacity and stronger binding to BNNS with the linker BP7. Thus, the BNNS-binding peptide provide a promising strategy for enhancing the immunostimulatory effect of CpG ODNs.

In chapter 3, another CpG ODNs delivery system using cationic polymer-functionalized BNNS has been developed. Polyethyleneimine (PEI) was coated on the surface of BNNS to achieve a positive surface charge, which facilitated the loading of negatively-charged CpG ODNs onto the BNNS. BNNS/PEI complexes greatly improved the cellular uptake efficiency of CpG ODNs. This further resulted in an enhanced IL-6 and TNF- α production from PBMCs compared to that of CpG ODNs directly loaded on BNNS. Most importantly, B class CpG ODNs loaded on BNNS/PEI complexes induced IFN- α , while neither free CpG ODNs nor CpG ODNs loaded directly on BNNS had this potential. It is thought that when the class B CpG ODNs were loaded onto the positively charged BNNS-PEI complexes, they formed the higher-order multimeric structure similar to class A CpG ODNs, and acquired the ability to induce the IFN- α .

In chapter 4, chitosan (CS) coated boron nitride nanospheres (BNNS) were used as carrier for the delivery of CpG ODNs. BNNS/CS complexes had positive zeta potential and exhibited a better dispersity and stability in aqueous solution than BNNS due to the CS coating. The BNNS/CS complexes greatly improved the loading capacity and cellular uptake efficiency of CpG ODNs due to their positive surface charge. The loading capacity of the CpG ODNs depend on the molecular weight (MW) of CS, which affected the positive charge density on the surface of BNNS. CpG ODNs loaded on BNNS/CS complexes significantly enhanced the IL-6 and TNF- α productions from PBMCs compared to that of CS/CpG ODNs complexes and CpG ODNs directly loaded on BNNS. We also found that molecular weight of the CS used for BNNS coating affected the cytokines induction through varying the strength of the condensation of the CpG ODNs. Surprisingly, different from PEI-functionalized BNNS, CpG ODNs loaded on BNNS/CS could not induce IFN- α production from PBMCs. However, the mechanism remains unclear and further investigation is under way.

Chapter 5 is a summary. In this study, we have successfully developed several novel delivery systems for CpG ODNs based on BNNS. These delivery systems improved the loading capacity and cellular uptake of CpG ODNs, and are proved to be effective in enhancing the immunostimulatory effect of CpG ODNs. Future work will be focused on the effect of chitosan on the Toll-like receptor 9 mediated IFN- α production.