氏名	Shireen Nigar
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学位論文題目	A study on the immune effects of synergistic oligodeoxynucleotide from probiotics （プロバイオティクス由来相乗型オリゴDNAの免疫特性に関する研究）
論文審査委員	主査　准教授　下里　剛士 教　授　鏡味　裕 教　授　真壁　秀文 准教授　米倉　真一 准教授　北澤　春樹（東北大学）

論　文　内　容　の　要　旨

Bacterial genomes span a significant portion of diversity, reflecting their adaptation strategies; these strategies include nucleotide usage biases that affect chromosome configuration also known as genomic DNA. The genomic DNA has recently been shown to elicit a highly evolved immune defense. This response can be selectively triggered for a wide range of therapeutic applications, including use as a vaccine adjuvant to immunotherapies for allergy, cancer, and infectious diseases. Here, I explore an immuno-synergistic oligodeoxynucleotide (iSN-ODN, named iSN34), derived from *Lactobacillus rhamnosus* GG (LGG) genomic sequences, that exhibits a synergistic effect on immune response to CpG-induced immune activation. Bacterial DNA is a potent stimulator of the host immune response that mediated by unmethylated CpG motifs which leads to abrogation of the immunostimulatory activity. Synthetic oligodeoxynucleotides (ODNs) which contains CpG motifs that are like those found in bacterial DNA stimulate a similar response and these immunomodulatory ODNs have various potential therapeutic effects to combat. Bacterial DNA is a PAMP that is mediated by members of the Toll-like family of receptors (TLRs). Here, I extend that observation by demonstrating the synergistic induction (in mouse splenocytes) of IL-6

by the combination of iSN34 with cell wall components of bacteria and fungi. Likewise, TLRs have been implicated in the recognition of the fungal pathogens.

Several components located in the cell wall or cell surface to fungi have been identified as potential ligands. The sequence of iSN34 was designed based on the genomic sequences of LGG. Pathogen-free mice were purchased from Japan SLC and maintained under temperature- and light-controlled conditions. I tested the effects of iSN34 exposure *in vitro* and *in vivo* by assessing effects on mRNA expression, protein levels, and cell type in murine splenocytes. I demonstrate that iSN34 has a significant stimulatory effect when administered in combination with CpG ODN, yielding enhanced interleukin (IL)-6 expression and production. I also observed that splenocytes pretreated with iSN34 and then co-stimulated with agonists for TLR1/2 (Pam_3CSK_4), TLR4 (LPS), or TLR2/6 (Zymosan) exhibited enhanced accumulation of IL-6. IL-6 is a pleiotropic cytokine that has been shown to prevent epithelial apoptosis during prolonged inflammation. My results are the first report of a bacterial-DNA-derived ODN that exhibits immune synergistic activity. The potent over-expression of IL-6 in response to treatment with the combination of CpG ODN and iSN34 suggests a new approach to immune therapy. Additionally, suggested that the combination of iSN34 with TLR1/2, TLR4, or TLR2/6 agonists may permit the induction of a potent immune response. This finding may lead to novel clinical strategies for the prevention or treatment of dysfunctions of the innate and adaptive immune systems.