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Review

Development of Mucosal Vaccines as Novel Preventive Methods against Infectious and Noncommunicable Diseases (NCDs): Contribution to a Society of Health and Longevity

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Keywords：Nasal vaccine, DNA Adjuvant, double DNA adjuvant (dDA), Dendritic cells, Prevention of infectious and noncommunicable diseases

Abstract：The surface layer of the mucosa, which in adult humans, is estimated to have a surface area over 200 times greater than that of the skin, is constantly in contact with foreign antigens, against which non-specific defense (innate immune mechanisms) and specific defense (acquired immune mechanisms) are activated. In the mucosal areas that are the entry points for foreign antigens, the efficient production and secretion of secretory IgA (SIgA) antibodies, which are the main form of specific defense, and mucosal vaccines, which are an efficient antigen delivery system for inducing and promoting antibody production, may offer a strategic tool for preventing not only infections, but also the development of lifestyle-related diseases. We have previously undertaken research and development of an immunostimulating agent (adjuvant) for use with mucosal vaccines based on mucosal immunity, particularly nasal vaccines that are administered to the nasal cavity. More specifically, a double DNA adjuvant (dDA) system that targets dendritic cells, which are one of antigen-presenting cells, was developed, and basic research was conducted using a number of different antigens, including a comparison of the immune response to these antigens when they were introduced into the nasal cavities of young and old experimental animals (mice). Of late, we have been working toward the development of a mucosal vaccine capable of preventing infection or lifestyle-related disease that can also be used in older people. In this review article, I will introduce the advantages of mucosal vaccines as well as recent findings in our group.

I. Introduction

Nearly all microorganisms including SARS-CoV-2, which emerged in 2019 are viruses or bacteria that enter the body via the respiratory mucosa of the oral and nasal cavities to cause infection and disease. According to the Ministry of Health, Labour and Welfare of Japan (2021), the number of serious cases and fatalities from COVID-19 by age increases dramatically from age 60 years and peaks among those in their 80s and 90s[1]. One major reason for this may be that the immunological defense mechanisms of older people are weakened by declining immunity in so-called "immunosenescence." Vaccines to induce and activate immunity may not be sufficiently effective under conditions of immunosenescence. Furthermore, according to a World Health Organization fact sheet, ischemic heart disease and cerebral stroke account for the largest number of deaths, and these chronic diseases involving vascular occlusion associated with inflammation are the most common cause of death

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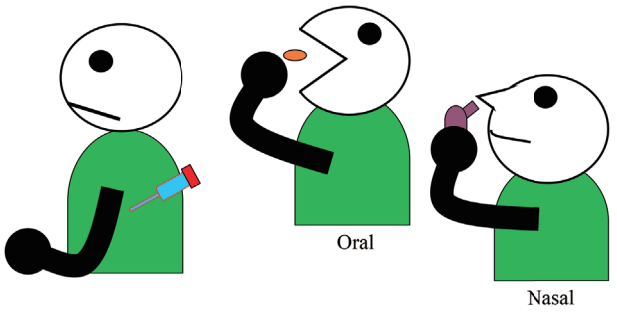

Immune Responses	Needle Vaccines	Mucosal Vaccines
Systemic	+++	+++
Mucosal	±	+++

Fig. 1 Immune responses in needle vaccines vs mucosal vaccines.（Schematic Diagram）

worldwide[2]. In Japan, heart disease other than hypertension and cerebrovascular disease occupy the second and fourth spots, respectively[3].

We have previously undertaken the research and development of a novel nasal adjuvant for use with mucosal vaccines based on mucosal immunity, particularly nasal vaccines that are administered to the nasal cavity. The double DNA adjuvant (dDA) system targeting dendritic cells was developed, and basic research using a number of different antigens was conducted, including a comparison of the immune response to these antigens when they were introduced into the nasal cavities of young and old experimental animals (mice). Of late, I have been working toward the development of a mucosal vaccine capable of preventing infection or lifestyle-related disease that can also be used in older people. In this review article, I would like to introduce the advantages of mucosal vaccines as well as my recent findings.

II. Double DNA adjuvant (dDA) system consisting of Flt3 ligand expression plasmid (pFL) and CpG Oligodeoxynucleotide 1826 (CpG ODN)

The mucosal surfaces of the gastrointestinal tract and respiratory organs, beginning with the oral and nasal cavities, are constantly exposed to foreign antigens and allergens. The mucosa is therefore the site of a unique biological response, the mucosal immune system, which is responsible both for detecting and excluding the invasion of pathogenic microorganisms[4] and for maintaining coexistence and balance with food antigens and the microbiome[5]. The mucosal immune system thus maintains homeostasis with the outside world while constituting the front-line defense that protects our bodies from external threats. SIgA antibodies are the main tool in this detection/defense system.

Conventional needle vaccines induce a systemic immune response, but the induction of antigen-specific SIgA antibodies in the mucosa is inefficient and problematic. Mucosal vaccines utilizing the mucosal immune system, however, are capable of the efficient induction of antigen-specific SIgA antibodies in the mucosa, and may also induce a systemic immune response (Fig. 1). Mucosal vaccines that act via the mucosa can thus act effectively on both levels of the immune system, preventing pathogenic microorganisms from invading the mucosa and expelling them from the body once they have invaded. Mucosal vaccines also offer additional advantages. Because they do not require injection by needle or syringe, they are not only painless, but also helpful in the prevention of secondary infection or medical accidents caused by the repeated use of such devices, and medical costs can be reduced because no medical waste is generated. Their simplicity also enables easy inoculations in times of disaster, when the social infrastructure may have broken down, meaning that these "next-generation vaccines" are a promising new tool that may benefit society. Normally, however, it is difficult to induce an immune response adequate for the prevention of infection by the mucosal administration of an antigen alone, and immune tolerance may sometimes be induced. The activation of the innate immune response is extremely important if vaccines are to be adequately effective[6], and adjuvants are responsible for this activation. Adjuvants activate antigen-presenting cells as a result of their recognition by Toll-like receptors (TLRs) and RIG-like receptors, which are innate immunity receptors, and as immunostimulating agents that induce an acquired immune response, they are essential bases for vaccines[7,8].

The mucosal adjuvant used in the dDA system is a combination of plasmids (pFL) expressing the cytokine Flt3 ligand, which is a growth factor for hematopoietic

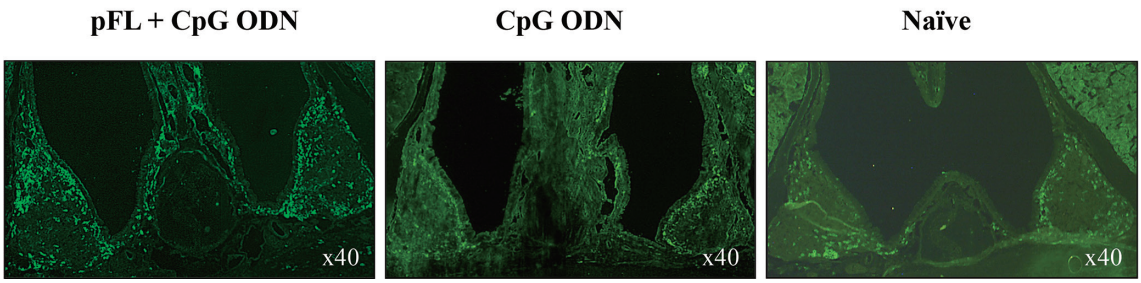

Fig. 2 Induction and increases of DCs in NALT by nasal dDA system.
Immunofluorescence staining of $CD11c^+$ DCs in NALT. BALB/c mice were immunized with OVA and plasmid encoding Flt3 ligand cDNA (pFL) and CpG (CpG ODN) or CpG ODN as nasal adjuvants. NALT taken from naïve mice were also stained as controls. Frozen sections of NALT were stained with biotin-conjugated, anti-CD11c (HL3) mAbs followed by HRP-conjugated streptavidin-Alexa Fluor 488®. The original magnification was 40 x. The picture is a typical example of results of immunofluorescence analysis of over 20 samples.

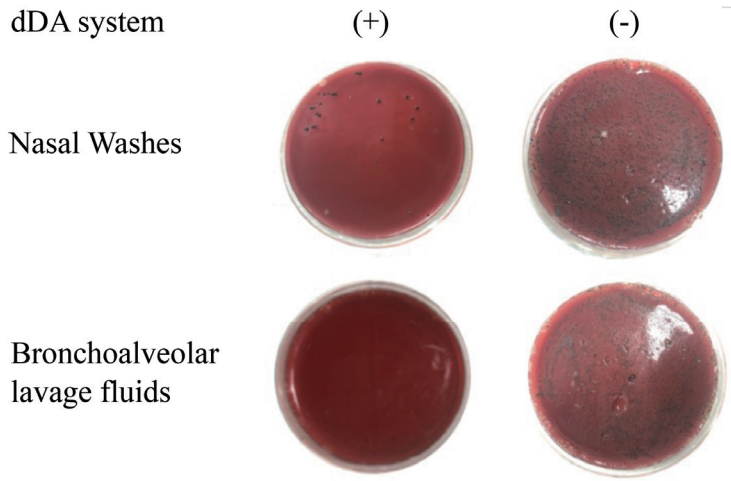

Fig. 3 Elimination of Pg live cells from upper and lower respiratory tracts by nasal dDA system.
Agar medium plates incubated with nasal washes or bronchoalveolar lavage fluids of $IgA^{+/+}$ mice given nasal FimA and dDA or FimA alone. One hundred μl of NWs and bronchoalveolar lavage fluids were spread on agar medium including kanamycin and were cultivated for 144 hours at 37℃ under anaerobic conditions.

and lymphoid progenitor cells, and the K-type CpG oligonucleotide 1826 (CpG ODN), which is a TLR9 ligand. For example, when this system was administered nasally to mice together with the OVA antigen, clear induction and growth of dendritic cells was observed in nasopharyngeal-associated lymphoid tissue (NALT), a mucosal immune-inductive tissue (Fig. 2), as was their activation. After the antigen stimulation of $CD4^+$ T cells from the spleens of vaccinated mice, a good balance of Th1- and Th2- type cytokines was induced, indicating that the dDA system is very safe[9]. In addition, when mice were administered FimA, the subunit protein of fimbriae located on the cell surface of the periodontal pathogenic bacterium *Porphyromonas gingivalis* (Pg) as an antigen, antigen-specific SIgA antibodies were efficiently induced in saliva, nasal washes, and bronchoalveolar lavage fluids. In addition, when mice were nasally infected with Pg after inoculation with the vaccine, the bacteria were effectively excluded from the upper and lower airways (Fig. 3). Experiments in IgA knockout mice also suggested that IgA antibodies are essential to this exclusion effect[10]. On the basis of these findings, we hope to develop a nasal vaccine for preventing aspiration pneumonia in the future using this dDA system to target dendritic cells.

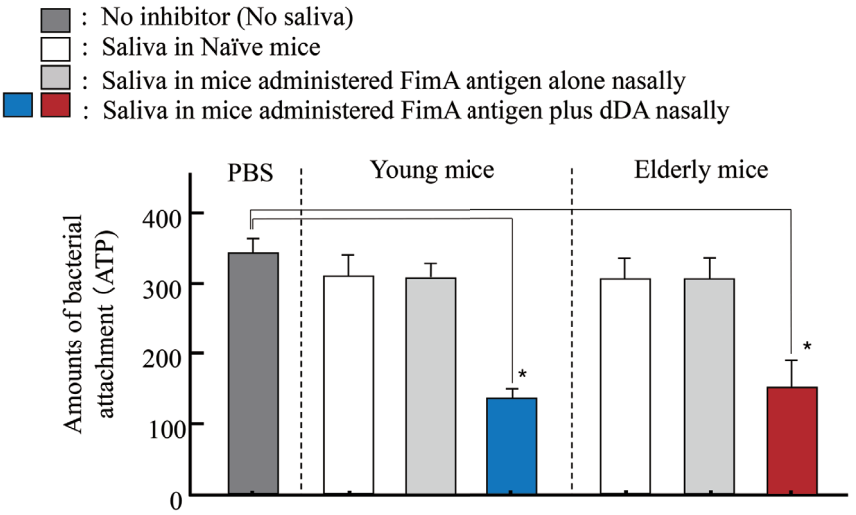

Fig. 4 Inhibitory effects of Pg live cells binding by salivary SIgA in experimental intraoral tooth surface model.
The inhibition of live Pg cell-binding to statherin by IgA-enriched saliva of 8- or 48-weeks-old mice nasally immunized with FimA plus dDA or FimA alone was examined. ATP amounts of live Pg cells were determined by luminescence using the Bac Titer-Glo Microbial cell viability assay. The numbers of 5 x 10^7 live cells are equivalent to 350 relative right unit (RLU) of the luminescence. The values shown are the mean ± SE (n = 5). *$p < 0.05$, when compared with mice group given FimA alone.

III. Anti-aging effects by nasal double DNA adjuvant

We conducted a comparative study of the numbers of dendritic cells in two mucosal inductive tissues, the Peyer patch (PP) and NALT, in naïve young mice and old mice in a steady state, and found that despite a marked reduction in the number of dendritic cells in the PP in old mice, in NALT, no reduction in the number of dendritic cells was found, which did not differ significantly from the number of dendritic cells in the NALT of young mice. We also compared the antigen-presenting capacity of NALT dendritic cells in young and old mice and found that the antigen-presenting capacities of the dendritic cells of the spleen and PP in old mice were significantly lower than those in young mice. For NALT dendritic cells, however, we observed the same level of $CD4^+$ T cell activation capacity in both old and young mice. This demonstrated that although aging/senescence is considered to be a universal, endogenous, and progressive phenomenon, the oral and nasal cavity immunity induced from NALT may constitute an immune system that is less affected by aging/senescence than are those from the PP or spleen, which constitute intestinal and systemic immunity[11].

We then administered FimA antigen and dDA nasally to young and old mice and used IgA-antibody-enriched saliva samples created by excluding IgM and IgG antibodies from subsequently collected mouse saliva to conduct an experiment investigating the effect of these saliva samples in preventing Pg adhesion to human saliva protein-covered hydroxyapatite beads as an experimental intraoral tooth surface model. The saliva samples from the young and old mice induced by the dDA system effectively suppressed the adhesion of Pg to the intraoral tooth surface model at around the same level (Fig. 4). This indicated that a nasal vaccine consisting of the FimA antigen using the dDA system effectively induced FimA-specific IgA antibodies in the saliva of old as well as young mice, and that these FimA-specific IgA antibodies were similarly effective in suppressing bacterial adhesion *in vitro*[12].

IV. Induction of atheroprotective IgM antibody by nasal double DNA adjuvant

In a previous study, when we nasally administered mice with phosphorylcholine (PC), which is found on the surface of pneumococcal bacteria, as the antigen and pFL as the adjuvant, we observed significant increases in anti-PC-specific IgA antibodies and IgM antibodies in bronchoalveolar lavage fluids, nasal washes, and serum. These antibodies also prevent plaque formation in atherosclerosis[13], and it has been suggested that they may be T15 idiotype antibodies, which are considered to be effective in preventing pneumococcal infections[14, 15]. When mice nasally administered this vaccine were infected with pneumococcus, the viable counts of pneumococcal bacteria in the lungs and nasal cavity at 12 hours after infection were significantly lower than those of the control group[16]. In addition, when we nasally administered the dDA system together with PC antigen to atherosclerosis

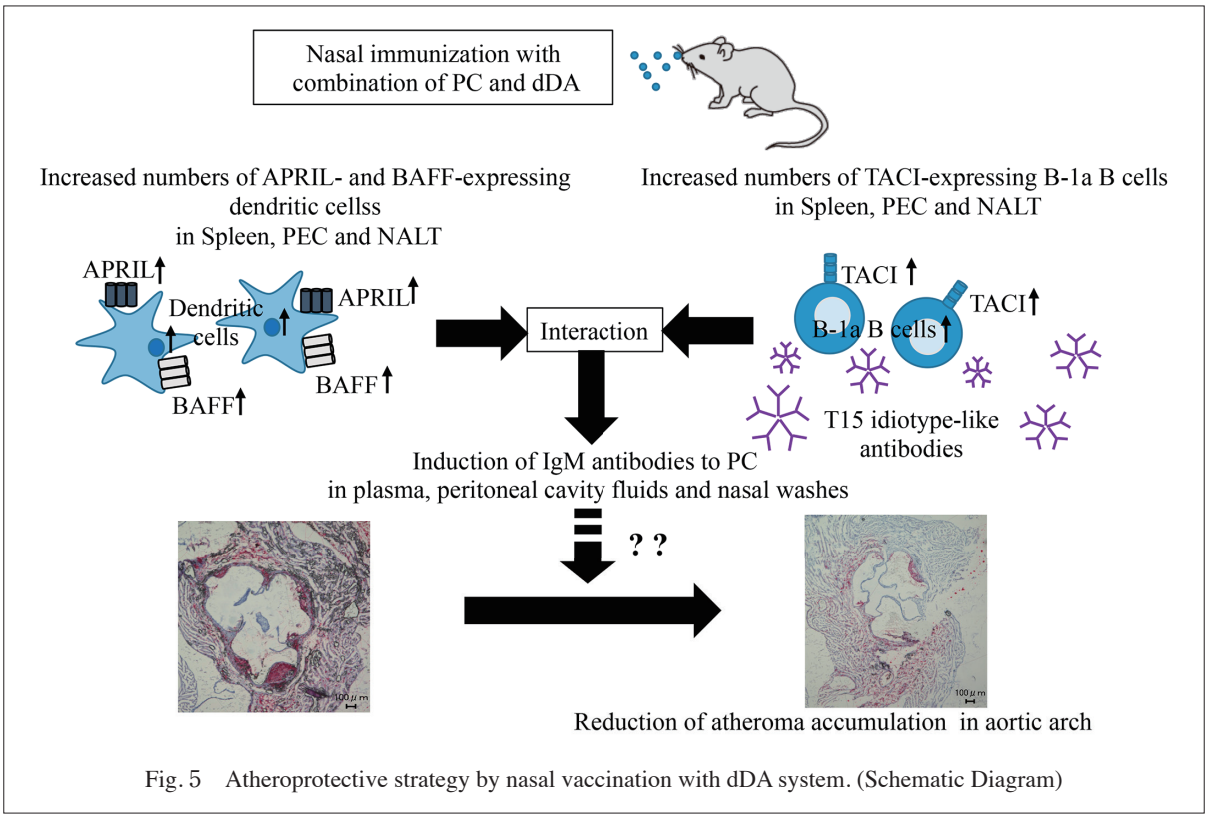

Fig. 5 Atheroprotective strategy by nasal vaccination with dDA system. (Schematic Diagram)

model (ApoE KO) mice, we showed that it had an inhibitory effect on plaque accumulation in the aorta through interactions between APRIL/BAFF-expressing dendritic cells and TACI-expressing B-1a B cells (Fig. 5). Although further studies will be needed to clarify whether IgM induced by nasal dDA system has atheroprotective effects, we suggest that this nasal vaccine using the dDA system may also serve as an atherosclerosis prevention tool[17].

V. Summary

The number of older people in Japan is projected to increase steadily until 2042, and by 2065, the proportion of the population aged 65 years and over is projected to exceed 38%. In Japan's "super-aged" society, eliminating the difference between the life expectancy and healthy longevity of older people and improving their quality of life are urgent issues. We will press ahead by further elucidating the oral and nasal cavity immune system based on NALT, which is less susceptible to the influence of immunosenescence. By making maximum use of tools such as secretory IgA antibodies in saliva, we hope to continue developing painless, safe nasal vaccines that prevent not only infection by viruses and bacteria that attempt to enter via the oral and nasal cavities, but also lifestyle-related diseases.

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Disclosure

The author declares that I have no conflicts of interest regarding this article.

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