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COMMENTARY

Cationic pullulan nanogel as a safe and effective nasal vaccine delivery system for respiratory infectious diseases

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ABSTRACT

The mucosal surfaces of the respiratory and gastrointestinal tracts are continuously exposed to countless beneficial and pathologic antigens. These mucosal surfaces are thus equipped with an immune system that is unique from those elsewhere in the body; this unique system provides the first line of immune surveillance and defense against pathogen invasion. The sophisticated immune induction machinery in the aero–digestive tract involves mucosa-associated lymphoid tissues, including nasopharyngeal- and gut-associated lymphoid tissues, for the generation of antigen-specific humoral and cellular immune responses. Consequently, nasal or oral immunization with an appropriate vaccine delivery vehicle prompts the induction of protective immunity in both the mucosal and systemic compartments, leading to a double layer of protection against pathogens. To harness the benefits of mucosal vaccines, various mucosal antigen delivery vehicles are under development, and a cationic cholesteryl-group-bearing pullulan nanogel (cCHP nanogel) has emerged as a potent nasal vaccine delivery system for the induction of protective immunity against respiratory infections.

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Introduction

In general, infectious pathogens including viruses and bacteria invade their hosts through the mucosal surfaces of respiratory and gastrointestinal tracts by inhalation and ingestion, respectively. Therefore, it is quite reasonable to protect the host from infectious diseases by inducing a specific immune response including neutralizing antibodies and cellular immunity at the entrance of these pathogenic microorganisms.[1] For this purpose, oral and nasal mucosal vaccines are attractive and effective immunization methods to elicit antigen-specific immune responses at mucosal surfaces. In particular, nasal vaccines have been shown to induce antigen-specific immune responses not only in the systemic compartment but also in the mucosal sites of the upper and lower respiratory tracts and reproductive tissues.[2] In response to this information, nasal vaccines targeting various infectious diseases are being developed worldwide.[3]

Nasal delivery of vaccines elicits a relatively lower immune response when the antigens are administered without delivery vehicles or adjuvants. Therefore, many strategies for nasal vaccines under development are aimed at identifying and developing appropriate mucosal adjuvants and antigen-delivery vehicles.[4] For example, an inactivated influenza nasal vaccine licensed in Switzerland and used during the 2001–2002 influenza season contained *Escherichia coli* heat-labile toxin as an adjuvant.[5] However, post-marketing surveillance revealed several cases of transient facial nerve paralysis (Bell's Palsy) as a side effect; consequently use of the vaccine was immediately discontinued due to safety concerns.[5] This example reminded the vaccine community of the close anatomical proximity of the nasal cavity to the central nerve system through the olfactory nervous system. Now, nasal vaccines developed for clinical use must incorporate mucosal adjuvants and transport systems that 1) safely and efficiently deliver antigen to nasal mucosal tissue and 2) prevent the deposition of adjuvants or vaccine antigens in the central nervous system.

Nanogel as a drug-delivery system for nasal vaccines

A nano-sized hydrogel nanoparticle (nanogel) has recently been proposed as a mucosal drug-delivery system.[6] The nanogel consists of cholesteryl-group-bearing pullulan (CHP) which, through hydrophobic association, assumes the structure of a spherical particle (Fig. 1).[6] Because protein is easily incorporated within the internal space of a CHP nanogel, its unique characteristics enable it to function as an artificial molecular chaperone.[7] Consequently, CHP nanogel was confirmed to be a useful protein-delivery vehicle.[6]

Like other mammalian cells, mucosal membranes are negatively charged.[8] To utilize charge-based interaction for the adherence of CHP to nasal epithelium and subsequent release of antigen, cationic types of CHP nanogel (cCHP) were a logical next step in the development of a nasal vaccine delivery system (Fig. 1).[9] In our studies, recombinant heavy chain from *Botulinum* type A neurotoxin (BoHc)—a receptor-binding but non-toxin-binding fragment of this neurotoxin—was effectively incorporated into the spherical cCHP particles.[10] Because of the

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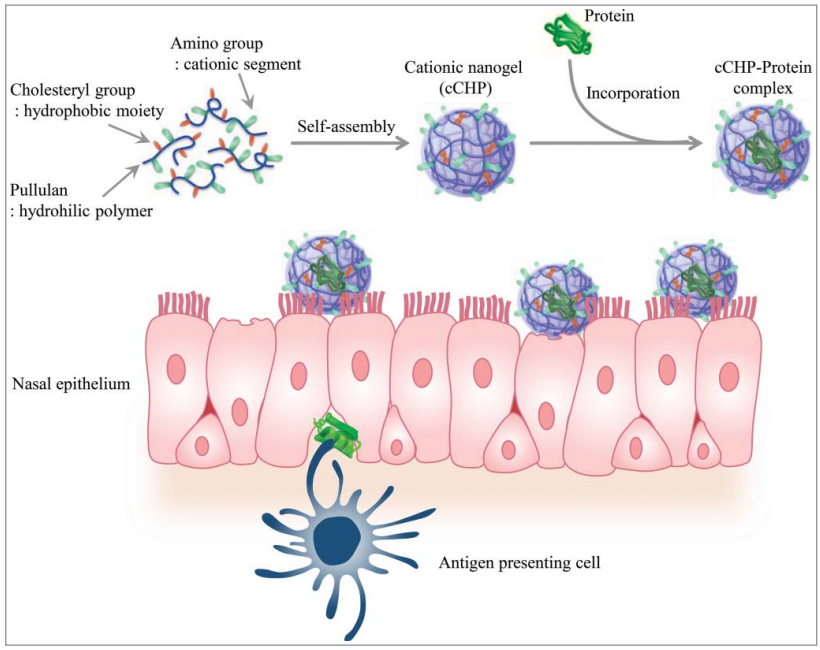

Figure 1. cCHP consists of a cholesteryl group–bearing pullulan (CHP) introduced cationic amino group. cCHP nanogel can encapsulate proteins in the interior space through hydrophobic interactions and are effectively retained in the nasal mucosa which are negatively charged after intranasal administration.

cationic nature of cCHP nanogel, cCHP–BoHc bound electrostatically to the negatively charged mucosal epithelium of the nasal cavity, including the nasopharynx-associated lymphoid tissue, an inductive tissue for the initiation of antigen-specific immune responses.[11,12] In addition, cCHP–BoHc was retained at the nasal mucosa for more than 48 hours after nasal administration,[10] and cCHP–BoHc remained in the nasal mucosa longer than BoHc alone.[10] BoHc antigens were released gradually from the cCHP nanogel, beginning within 1 hour after nasal administration, and antigen release continued over a 12-hour period.[10]

Dendritic cells located beneath nasal epithelial cells then internalized the cCHP-released BoHc antigen.[10] Approximately 40% of the dendritic cell population located below the basement membrane of the nasal epithelium and lamina propria of the nasal cavity acquired BoHc antigen by 6 hours after nasal administration of cCHP–BoHc.[10] In contrast, 2% or fewer dendritic cells engulfed antigen after nasal administration of naked BoHc. Furthermore, nasal vaccination of mice with cCHP–BoHc nanogel elicited high titers of neurotoxin-neutralizing serum IgG and secretory IgA (SIgA) antibodies, which provided protective immunity against lethal systemic and mucosal challenge with the neurotoxin.[10] Moreover, cCHP nanogel delivery did not lead to the deposition of vaccine antigen into the olfactory nervous system.[10] Taken together, these results support the use of cCHP nanogel as an antigen delivery system for adjuvant-free nasal vaccination that induces effective immune responses in both the systemic and mucosal compartments.

Development of a nanogel-based nasal vaccine against pneumonia

Given the potency of cCHP nanogel as a nasal vaccine delivery system, we next aimed to develop a nasal vaccine against respiratory infectious disease. *Streptococcus pneumoniae* causes serious upper respiratory tract infections worldwide that often lead to deaths due to bacterial pneumonia, primarily among young children and the elderly.[13] Specifically, pneumonia accounts for 16% of all deaths of children younger than 5 years, and the World Health Organization estimated that 1 million children died of pneumonia in 2015.[14] In addition, the annual incidence of pneumonia in people older than 65 years is four times that of younger people, with a correspondingly higher rate of hospitalization.[15]

Currently, two types of pneumococcal vaccines are available—pneumococcal polysaccharide vaccine (PPSV) and pneumococcal polysaccharide conjugate vaccine (PCV)—both of which are administered intramuscularly.[13] These vaccines are based on the polysaccharide capsule, which expresses the major virulence factors of *S. pneumoniae*.[16] In PPSV, the antigens, which are purified capsular polysaccharides from various serotypes of *S. pneumoniae*, elicit antigen-specific responses in a T-cell-independent manner. In contrast, in PCV, capsular polysaccharides are conjugated with the carrier protein CRM197, a non-toxic mutant of diphtheria toxin; PCV contains an aluminum phosphate adjuvant and induces T-cell-dependent responses.[17] Although these vaccines have potent immunogenicity against the most prevalent serotypes that cause bacterial pneumonia, a recurrent clinical concern involves pneumococcal serotype replacement. The current polysaccharide-based multivalent vaccines induce protective immunity against and suppress infections due to *S. pneumoniae* isolates of the same serotypes as the vaccine antigens and not other serotypes.[18-21] In this way, the current multivalent vaccine promotes a vicious cycle of pneumococcal serotype replacement. To overcome this problem, vaccines containing one or more universal antigens that are expressed by all serotypes of *S. pneumoniae* and thus induce protection against all isolates of *S. pneumoniae* are desired.

The pneumococcal surface protein A (PspA) antigen is a highly immunogenic and highly conserved surface protein that is expressed on all clinical isolates of *S. pneumoniae*. Because it thus can induce cross-reactive immune responses among different strains, PspA is a promising candidate antigen for the development of a next-generation pneumococcal vaccine.[22,23] Nasal administration of a fusion protein comprising PspA and flagellin from *Vibrio vulnificus* induced antigen-specific IgG and IgA both in serum and at mucosal surfaces and provided efficient protective immunity in mice against lethal challenge with live *S. pneumoniae*.[24] In addition, co-administration of PspA antigen and IL-12 as a nasal adjuvant enhanced PspA-specific IgG and IgA responses, with increased protection from nasal carriage.[25] In addition, nasal immunization with chitosan–DNA nanoparticles that express PspA elicited protective immunity against nasal colonization by *S. pneumoniae*.[26] Furthermore, an antigen-delivery method has been developed that targets claudin-4, a major cell-adhesion molecule in tight junctions that is highly expressed on the epithelium of nasopharynx-associated lymphoid tissue.[27] PspA–C-CPE, a fusion protein comprising PspA and a C-terminal fragment of *Clostridium perfringens* enterotoxin (C-CPE), which binds claudin-4, efficiently attached to nasopharynx-associated lymphoid epithelium after nasal administration to mice.[27] In addition, PspA–C-CPE effectively induced antigen-specific immune responses, and PspA-specific antibodies were elevated not only as IgG in the serum and bronchoalveolar lavage fluid but also as IgA in nasal washes and bronchoalveolar lavage fluid. Consequently, mice nasally vaccinated with PspA–C-CPE were protected against pneumococcal infection.[27] In addition, nasal immunization of PspA with mucosal adjuvants, such as a plasmid-expressed Flt3 ligand and CpG oligodeoxynucleotides, yielded PspA-specific SIgA in aged or pregnant mice.[28] After nasal vaccination, bacterial colonization was inhibited even in 2-year-old (that is, aged) mice and in pups born to vaccinated dams.[28,29] These results support PspA as a promising antigen candidate for an *S. pneumoniae* vaccine that is likely to be effective not only in adults but also in children and the elderly.[30,31]

Capitalizing on the effectiveness of PspA as a vaccine antigen for protection against all types of *S. pneumonia*, a cCHP nanogel containing PspA (cCHP–PspA nanogel) has been developed as a new nasal vaccine aimed toward clinical use.[32,33] When cCHP–PspA nanogel was nasally administered 3 times at 1-week intervals to mice, antigen-specific IgG levels were significantly elevated in the serum and bronchial fluids, and antigen-specific SIgA levels were increased in nasal fluids. In

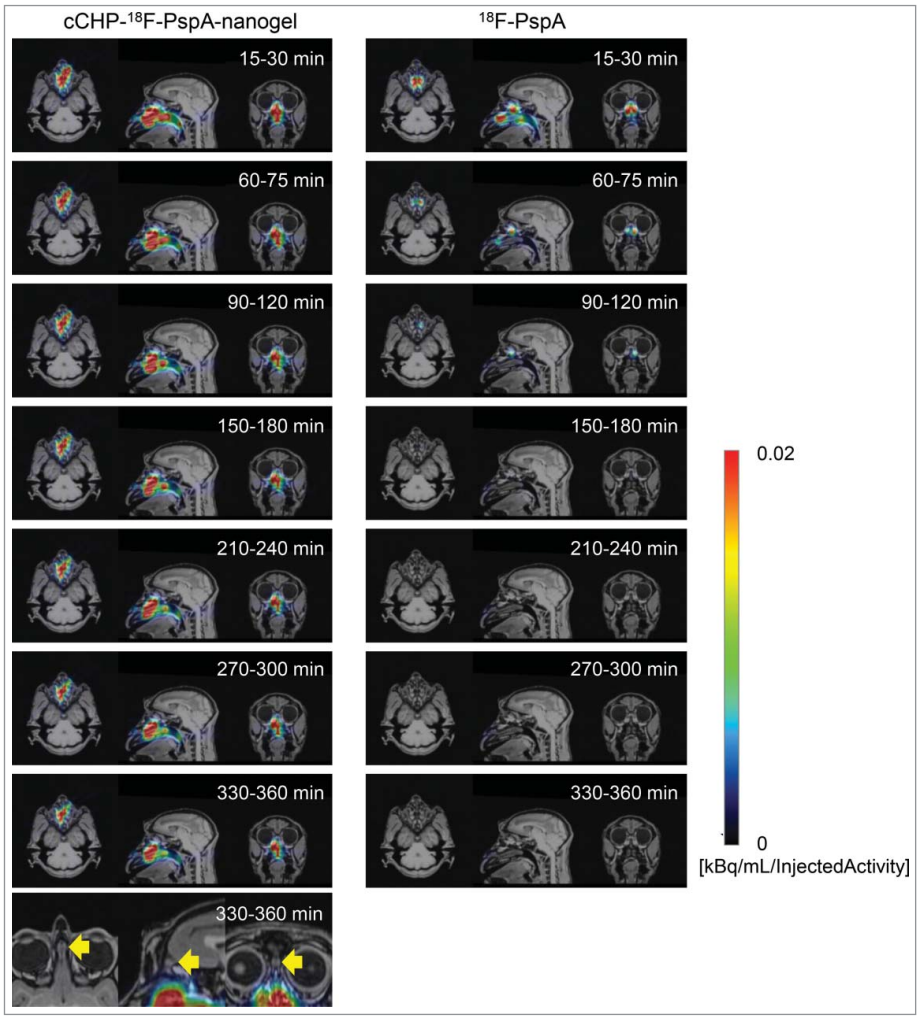

Figure 2. Distribution of nasally administered ^{18}F-labeled PspA, a surface protein of S. pneumoniae, in cCHP nanogels or PBS by positron emission tomography and magnetic resonance imaging. No deposition of ^{18}F-labeled PspA in olfactory bulb and brain as indicated with arrows.

contrast, administration of PspA antigen alone failed to induce any antigen-specific antibodies. PspA antigen was efficiently delivered to nasal epithelium and subsequently taken up by dendritic cells for the initiation of an antigen-specific immune response in the mice immunized with cCHP–PspA nanogel but not those given PspA antigen only. Consequently, bacterial growth was suppressed both in the lung and nasal cavity of the mice vaccinated with cCHP–PspA nanogel, which then were protected against lethal challenge with *S. pneumonia*. The protective immunity elicited through cCHP–PspA nanogel was accompanied by the production of both Th2- and Th17-type cytokines by antigen-specific $CD4^+$ T cells, a feature that is known to be associated with protective immunity against *S. pneumonia*.[32]

To advance the application of the cCHP–PspA vaccine in humans, non-human primates were nasally immunized with the cCHP-based pneumococcal vaccine.[33] When a cCHP–PspA nanogel was nasally administered to rhesus macaques 5 times at 2-week intervals, serum levels of PspA-specific IgG were significantly elevated and then gradually decreased over a period of 8 months. In addition, the production of PspA-specific SIgA was induced in both nasal washes and bronchoalveolar lavage fluids and then decreased in the same manner as did PspA-specific IgG in the serum. When these macaques received a dose of cCHP–PspA nanogel nasally at 9 months after the final immunization, PspA-specific IgG and IgA antibodies were rapidly boosted to higher levels than those after primary immunization, indicating that nasal vaccination with cCHP–PspA nanogel effectively generated memory responses. In addition, these immune responses were accompanied by Th2 and Th17 cytokine production. Furthermore, the cCHP–PspA nanogel induced PspA-specific antibodies with neutralizing activity against *S. pneumoniae*.[33] In a positron emission tomography (PET) study combined with MR imaging to monitor the deposition and fate of vaccine antigens in the nasal cavity, nasally administered ^{18}F-labeled PspA (^{18}F-PspA) showed prolonged retention in the nasal epithelium (that is, for as long as 6 hours), compared with PspA antigen alone (Fig. 2). Furthermore, particularly important is the fact that the nanogel-delivered vaccine antigen did not deposit into the olfactory bulbs or brain in the macaques. Therefore, cCHP–PspA nanogel exhibits promising characteristics of a safe and effective nasal vaccine candidate for the prevention and control of pneumonia.[33]

Application of cCHP for nasal vaccines against non-infectious diseases

Recently, the cCHP nanogel system was adopted for the control of lifestyle-related diseases, including obesity and hypertension.[34, 35] For example, a vaccine formulation has been created in which PspA is fused as a carrier protein to recombinant ghrelin, a peptide hormone produced in the stomach (ghrelin–PspA). Because ghrelin increases appetite (and thus increases food intake) and decreases energy expenditure,[36] antibody against ghrelin theoretically could restore the balance between food intake and energy expenditure, leading to homeostasis and preventing obesity. Indeed, nasal administration of ghrelin–PspA with cyclic diguanosine monophosphate as an adjuvant increased antigen-specific serum IgG levels and decreased body weight in mice with diet-induced obesity.[34] Therefore, as a novel antigen-delivery vehicle, cCHP nanogel has great potential not only for nasal vaccines against infectious diseases but also for therapeutic vaccines against lifestyle-associated disorders.

Conclusions

cCHP nanogel is a promising vaccine delivery system because of its safety and its efficacy in inducing antigen-specific protective immunity. Although cCHP nanogel itself lacks biological adjuvant activity, it effectively initiates the induction of antigen-specific immune responses in both the systemic and mucosal compartments by efficiently delivering antigens to nasal dendritic cells. Furthermore, PET analysis combined with MR imaging confirmed that nasally inoculated vaccine antigens did not migrate into the olfactory bulbs and brains of mice and macaques, suggesting that cCHP nanogels can be incorporated into safe nasal vaccines for human use. cCHP nanogels might be combined with various candidate vaccine antigens to achieve next-generation nasal vaccines for infectious and lifestyle-associated diseases.

Abbreviations

BoHc	*Botulinum* type A neurotoxin fragment
cCHP	Cationic formulation of CHP nanogel
C-CPE	C-terminal fragment of *Clostridium perfringens* enterotoxin
CHP	Cholesteryl-group-bearing pullulan
PCV	Pneumococcal polysaccharide conjugate vaccine
PET	Positron emission tomography
PPSV	Pneumococcal polysaccharide vaccine
PspA	Pneumococcal surface protein A
SIgA	Secretory IgA

Disclosure of potential conflicts of interest

No potential conflicts of interest exist.

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