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Anti-caries vaccine based on clinical cold-adapted influenza vaccine: A promising alternative for scientific and public-health protection against dental caries

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ABSTRACT

Dental caries remains one of the most pervasive infectious disease around the world. Protection against dental caries can be achieved experimentally by eliciting salivary IgA targeting surficial antigens of *S. mutans*, however, no such a vaccine has been launched for human use yet. Live vectored vaccines hold the greatest feasibility to induce potent and long-lasting immunity in the host. The FDA approved intranasal cold-adapted influenza vaccine has been used in clinical settings for many years. The vaccine can not only induce broad adaptive immune responses especially mucosal immunity, but the member strains can also circumvent existing immunity, presenting promising candidates for live vectored anti-caries vaccine. Moreover, the genetic techniques for modification of cold-adapted influenza viruses are well developed and widely applicable. Thus, we hypothesize that effective anti-caries vaccine can be developed with the backbone of cold-adapted influenza viruses by inserting specific antigenic identifier sequences of *S. mutans* into the viral genome, which is anticipated to protect against dental caries in humans with easy inoculation. The immune efficacies of recombinant cold-adapted influenza viruses expressing exogenous antigens have been verified by *in vivo* experiments for multiple infectious diseases, giving us great confidence to validate the safety properties and protection effect with this chimeric vaccine in animals or even humans. Existing data suggests that the live anti-caries vaccine may help improve public oral health by controlling the caries disease itself.

Introduction

Dental caries is a common infectious disease that results in localized dissolution and destruction of the calcified tissue. The disease usually develops slowly and occurs throughout the entire life, affecting children, adolescents, as well as adults [1]. The World Health Organization (WHO) has classified it as one of the most pervasive diseases of humanity, causing serious health hazards and heavy economic burden [2]. Furthermore, epidemiologic studies have noted that after many years of declining caries rates, there has been a recent increase in childhood caries in some industrialized countries, despite easier access to public and private health measures [3]. Not to mention the magnified population with dental caries in developing countries due to economic deprivation or bad social infrastructure [4]. Vaccines have long been attractive for broad-based public health coverage of infectious diseases, however, no ideal anti-caries vaccine has been developed [5].

Over the past decades, a large body of experiments have indicated *Streptococcus mutans* (*S. mutans*), which has long been considered the major causative agent of dental caries, as an effective target for preventing dental caries in animals, or even in small-scale human trials [6,7]. In fact, the specific immune defense against cariogenic *S. mutans* is mediated largely by salivary secretory IgA (S-IgA) antibodies, which are the primary immune component of salivary milieu and generated from the common mucosal immune system [8]. Given the reality that the S-IgA is more potently induced by mucosal immunization than parenteral immunization, various strategies of mucosal inoculation are currently under development, including use of intact antigenic proteins, derived recombinant or synthetic peptides, and DNA-based active

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vaccines with novel mucosal adjuvants [5]. However, not one vaccine has been brought to the market due to the low ability to induce and maintain enough protective antibodies in oral fluid.

Live vector vaccines hold considerable feasibility to elicit a potent and long-lasting immune response, which could be achieved by inserting immunogenic genes into live, infectious, but non-disease-causing viruses or bacteria. Generally, these vaccines possess several potential advantages than traditional vaccines, such as good expression of the inserted constructs, capacity for multiple gene inserts, availability of various strains, and strong immunogenicity of vector itself [9]. For now, live anti-caries vaccine based on *Salmonella typhimurium* has been developed to induce salivary IgA [10]. However, the vector is orally administrated and has to go through the harsh acidic environment in the stomach before it arrives at the inductive intestinal site risking potential vector damage as it travels from the mouth to the intestinal mucosa. To bypass this concern, a significant amount of vaccine is needed, which is not ideal as the high concentration of vector may be toxic to the host. Moreover, the preexisting immune response to the vector or the wild-type microorganism due to previous infection could eliminate the vector or transfected cells, diminishing the vaccinal efficiency [11].

Cold-adapted influenza virus (CAIV) has been used as a live vector for some infectious diseases, and data shows that immune responses against the desired antigens has been observed *in vivo*, indicating that CAIV is a promising alternative vector for an anti-caries vaccine [12,13]. Practically speaking, CAIV is the only FDA approved live attenuated influenza vaccine (LAIV) for humans, having a strong safety record in clinical settings for more than a decade, and the ability to elicit broad adaptive immune responses, especially mucosal immunity. Genetically manipulated influenza viruses have the potential to elicit protective immune responses against the foreign pathogen at both mucosal and systemic level. Moreover, the reversed genetic techniques for modification of cold-adaptive influenza virus are well developed [14]. Conveniently, the CAIV spray is administered through the relatively easy and painless intranasal route, which is conceived as a more attractive strategy for inducing anti-caries S-IgA immune response, due to its fertile mucosa lymphoid tissue and tropism to transport effector immune factors to salivary glands [15]. Despite this information, there is no reported data about cold-adapted influenza virus vectored anti-caries vaccine tested *in vivo*.

Hypothesis

Given the superior safety character, strong immunogenicity, and manufactural feasibility of cold-adapted influenza viral vector, we hypothesize that clinical anti-caries vaccine could be developed by inserting particular antigenic sequences of *S. mutans* into the viral genome. The new vaccine is anticipated to produce abundant antigen-specific IgA in the mouth after intranasal inoculation, thus providing effective protection against dental caries in humans.

Evaluation of hypothesis and discussion

Feasibility, versatility and immunogenicity of the recombinant caries-CAIV vaccine to S. mutans

The virulence of mutans streptococci (MS) depends on the ability to adhere and accumulate on dental surfaces, which is closely related with several cell-surface antigenic proteins, including streptococcal surface adhesion protein Pac, the glucosyltransferases (GTFs), and glucan-binding protein (GBP) [16]. Luckily, these virulent components are vulnerable to blocking by salivary IgA through complementary antigen-antibody bind medicated by small antigenic epitope or identifier on an antigen. Besides, these conservative antigenic determinants are proved to be effective immunogens for eliciting antigenic-specific antibodies as native protein [17]. In the past decades, quantity of antigenic epitopes within Pac, GTFs and GBP have been precisely identified by scientists, some sequences even demonstrated to be cross-reactive between different cariogenic bacteria, and their potential to generate protective immunity against *S. mutans*-associated dental caries have been well demonstrated [18–20], thus providing substantial immunogenic sequences for constructing live vaccines inducing *S. mutans* special antibodies as needed.

Single and multiple foreign genes have been correctly manipulated to insert into cold-adapted influenza virus (mainly type A influenza virus) genome without interfering with the viral replicability, and independent foreign peptides can be sustainably coded with virus replication in eukaryotic host cell [13,12]. The same strategy can be applied to produce *S. mutans* epitopic polypeptides. Though the influenza viral genome is small, the largest polypeptide that can be successfully expressed from influenza vector is about 250 amino acids long. This is far greater than the average size of the known *S. mutans* epitopes, thus it is possible to insert repetitive sequences, or design broader-spectrum vaccines for dental caries by synthesizing short epitopes together [21]. Interestingly, the immunogenicity of diepitopic vaccines is demonstrated to be significantly better than oligoepitopic peptide for combating dental caries [22].

Like the wild-type vector, recombinant cold-adapted influenza virus can not only sensitize the innate immune system, facilitating the submission of antigens to target lymphoid cell or organ, but it can also stimulate densely dispersed germinal centers in mucosa of the upper respiratory tract. This results in a continuous source of antibody-producing B cells, which can be activated and migrated to the salivary glands through the network of the mucosal immune system. Therefore, a more potent and long-lasting immune response is anticipated than in other nonreplicable vaccines [23]. The negative outcome is that the activated immune system will mostly result in a memory immune response against the same vector in inoculators, thus blemishing the intensity and duration of exogenous antigen. However, for influenza vectors with great antigenic diversity, it can be easily resolved by adopting influenza virus with mutative antigens or other types from the quadrivalent LAIV [24]. Additionally, though the long-time mucosal immunity elicited by LAIV may also interfere with replication of heterogeneous influenza viral vector, previous studies showed it doesn't compromise efficacy of new LAIV vaccine because the immune response remains high enough [25].

Safety property

In developing a vaccine for human use, safety concerns are very important, especially for targeting a non-lethal disease as dental caries. CAIV strains contain HA and NA gene segments derived from the currently circulating wild-type strains along with six gene segments, PB1, PB2, PA, NP, M, and NS, from a common master donor virus (MDV) [26]. It is rare for the virus in cold-adapted influenza vaccine to undergo genetic reversion or mutation into a pathogenic influenza strain due to its temperature-sensitive phenotype affirmed to be controlled by multiple mutations across the viral genome of MDV [27]. As to the recombinant caries-CAIV vaccine, optimally, it should not compromise the crucial safety characteristics of CAIV strains, which is quite predictable when the antigenic epitopes are inserted into the NA or HA segments without disturbing the MDV property [12]. More excitingly, previous studies have shown that recombinant CAIV vaccines with insertion in the NS segments present a similar attenuated phenotype as the wild-type [28].

Another concern about the recombinant caries-CAIV vaccine is the possibility of transmitting through the air and its potential to cause disease in particular populations. It has been well established that the cold-adapted influenza virus is poorly transmissible because most infections are not symptomatic, decreasing the likelihood of viral spread via a cough or sneezing [29]. In any event, choosing epitopes totally free of interaction with antigens of other host tissue [30] and further

improving the attenuated property of the vector through reverse genetic approaches [31] will minimize possible hazard and expand applicable use in the population.

Efficiency in vivo

An inverse relationship between salivary IgA antibodies to *S. mutans* and its early oral colonization, or the colonized individual's caries experience, has also been reported [32], thus a higher baseline level of salivary IgA is required for maintaining oral health from dental caries. Previous vaccinal zooperies based on recombinant cold-adapted influenza virus have been conducted in some institutions and yielded protection in animals due to a potent humoral, mucosal, and cell-mediated immune response [12,33,34]. Moreover, studies have demonstrated that cold-adapted influenza vector could particularly prime the immune system of animals or man for a fast and more vigorous immune response for a booster vaccine given months and even years later [13,35], and this unique feature should be further explored to induce robust antibody responses against caries in humans.

Potential clinical benefit

The FDA approved cold-adapted vaccine, though forbidden to use in infants and adults elder than 49 years old, the caries-CAIV vaccine can still provide protection to vast population [36]. Children are the most susceptible population for caries [1], and CAIV is demonstrated safe for children as young as 2 years old, which is earlier than the "window of infectivity" as *S. mutans* permanently colonize in the human mouth [37]. Interestingly, some parallel observation showed that young children who are free of *S. mutans* infection naturally during the "window of infectivity" remain undetectably infected for several years till eruption of the secondary dentition [38]. This suggests that a longer-term protection from colonization of *S. mutans* may be observed by immunization with this new anti-caries vaccine if administered in early childhood. Moreover, horizontal transmission of mutans streptococci between members of a group (e.g., family members of a similar age or students in a classroom) have been reported [37], thus immunization may produce herd protection in grouped populations.

Currently, the strategies for caries prevention include fluoride supplements, regular dental care, sealants and oral health education, which are mostly effective but not available to everyone. Economic, behavioral, or cultural barriers further impede their use in some populations, resulting in the epidemic of dental disease on a global level [39]. In addition, the treatment of replacing decayed parts of the tooth with proper restoration is unable to eradicate caries or pathogenic bacteria on other teeth, and the situation is even worse for people with dental phobias who are unable to cooperate in the treatment. However, CAIV-caries vaccine can induce IgA antibodies in saliva and protect each tooth in the mouth. Besides, the fee for cold-adaptive influenza virus based anti-caries vaccine is relatively inexpensive, because of the easy production process [40] and multiple manufacturing plants worldwide for LAIV, including low- and middle-income countries. Furthermore, the new vaccine can be used as an intranasal spray, which is easy, painless, and does not require professional staff, making it more acceptable for people, especially children.

Testing the hypothesis

Generation and replication of recombinant caries-CAIV vaccine will refer to the protocol described by Pérez-Girón. etc. [41], and replace the insert sequence with antigenic epitopes of *S. mutans*. To validate the stable expression of the specific antigenic epitope, the virus can be passaged in MDCK cells, and the target antigen can be detected by Western blotting. For evaluating the safety property of LAIV, the titer of recombinant caries-CAIV can be tested after incubation in MDCK cells at different temperatures [12].

Rodent dentition represent an excellent model for studies of dental caries. To evaluate the anti-caries effect of vaccines in animals, specific pathogen-free (SPF) mice and rats could be adopted, and intranasally dosed in a prime-boost way as Li et al. etc. with different CAIV vectors [13]. Particular IgA antibody levels in serum and saliva, *S. mutans* colony level in the oral cavity, as well as caries scores can be detected following the previous study [42].

Referring to the FDA approved cold-adapted vaccine, the primary inclusion criteria for phase I clinical testing could include healthy people ranged 2–49 years old, excluding people with egg allergies, pregnant women, immunocompromised individuals, people with asthma and others underlying medical conditions [36]. Informed consent should be obtained from the subjects that participated in these studies, and the subjects will be randomly assigned to control and inoculation groups balanced for age, sex, and oral condition. All subjects included should not have any active caries lesions prior to the first dosage and boost inoculation. Salivary IgA and colony level of *S. mutans* in supragingival plaque can be detected consulting former research [7]. Caries experience index (DMFT) will be recorded according to WHO standards [43]. All data will be expressed as quantity change from baseline to a certain follow up period and analyzed by overall one-way ANOVA followed by Tukey's test for group comparisons.

Conclusion

Clearly, dental caries is still a prevalent disease within the population. Thus an effective immunization strategy to control the infectious disease is urgently needed. The cold-adapted influenza viruses, which have been used in clinics for more than 15 years, hold a great potential for developing valid vaccines against dental caries. In a greater context, the use of recombinant cold-adapted influenza vaccine can prevent dental caries in an easy and inexpensive way. Ultimately, only provable superior clinical immunogenicity and safety *in vivo* testing can certify the usefulness of an anti-caries vaccine formulation for public health applications.

Conflict of interest

The authors declare no conflict of interest.

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