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Vaccine Targets Expanding from Infectious Diseases to Non-infectious Diseases

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***Abstract*—** *Many infections have been overcome by the spread of vaccines. Vaccines are the most cost-effective way of preventing infectious diseases. The main targets of vaccines are infectious diseases. For example, influenza and pertussis are familiar infectious diseases. Although conventional vaccines against influenza and pertussis have achieved some success in controlling these diseases, these infectious diseases have not yet been completely controlled. More effective vaccines against these infectious diseases are required to be developed. On the other hand, the possibility of the vaccines against non-infectious diseases such as Alzheimer's disease, high blood pressure, diabetes and Parkinson's disease have been shown. Patients with Alzheimer's disease have increased with the aging of the population. Overcoming the Alzheimer's disease is important for us to send better old age. Since the possibility of the vaccine for Alzheimer's disease has been shown, so many studies on vaccines for Alzheimer's disease have been carried out. The development of the Alzheimer's disease vaccine will accelerate the development of vaccines for other non-infectious diseases like high blood pressure, diabetes and Parkinson's disease.*

***Keywords*—** *Vaccine, Infectious Disease, Non-infectious Disease, Influenza, Pertussis, Alzheimer's disease.*

I. INTRODUCTION

Since the vaccine against smallpox by Jenner in the 18th century, many vaccines have made it possible to prevent infectious diseases. The name vaccine is said to be derived from the Latin name Vacca of the cochlea from which Jenner collected the material of the vaccine. On the other hand, antibodies have been used as a treatment for infectious diseases since Serum therapy was developed by Kitasato in the 19th century. Thereafter, antibody treatment has spread the targets from infectious diseases to non-infectious diseases. For example, antibody therapeutics for arthritis are in the top 10 of the drugs that are sold all over the world. Sales of antibody therapeutics for non-infectious diseases far exceed sales of antibody therapeutics for infectious diseases.

On the other hand, the main targets of vaccines are still infectious diseases. Although influenza and pertussis have been controlled to some extent by vaccines, they have not yet been completely eliminated. There is a strong demand for the development of more effective vaccines against these diseases. Under such circumstances, development of vaccines for non-infectious diseases such as Alzheimer's disease (AD), high blood pressure, diabetes and Parkinson's disease are also under development [1]-[4].

With the aging of the population, patients with AD has increased. Since the possibility of developing AD vaccine has been shown, many studies on AD vaccines have been conducted worldwide. AD is a serious non-infectious disease. The development of AD vaccine will also accelerate the development of vaccines for other non-infectious diseases like high blood pressure, diabetes and Parkinson's disease. It is important from the viewpoint of quality of life and medical economics that vaccine targets spread from infectious diseases to non-infectious diseases.

This article takes influenza and pertussis as examples of infectious diseases and AD as an example of non-infectious diseases. And vaccines and vaccine candidates against these infectious diseases and non-infectious disease are introduced.

II. INFLUENZA VACCINE

Influenza remains a serious ongoing threat to public health. Influenza is an infectious disease that plagues us every year [5]. And also, the new strains of influenza that occurs at intervals of several decades have caused many victims [6]. In either case, vaccination is the most cost-effective public health measure to prevent disease and mortality caused by influenza virus infection. Embryonated chicken egg or cell culture is used for production of influenza vaccines [7]. In case of pandemic influenza, the embryonated chicken egg cannot be used for vaccine production because chickens are influenced by pandemic influenza virus. As a measure to prevent pandemic influenza, cell culture system of influenza vaccine has been promoted worldwide. However, current influenza vaccines cannot suppress mutant virus and cannot protect invasion from respiratory mucosa [8],[9].

Recently, a novel therapeutic drug baloxavir marboxil that inhibit cap-dependent endonuclease, an enzyme essential for viral replication has been put on the market [10]. Under these circumstances, in order for the influenza vaccine to show its presence, it is necessary for novel vaccines to have such features as to protect against mutant viruses and to prevent invasion of viruses from respiratory mucosa.

Influenza A and B viruses accumulate mutations due to no proof-reading activity. When these mutations occur in the HA of influenza virus surface protein which is the main component of current influenza vaccine, they lead to antigenic drift, which over time results in escape from earlier immune responses. Influenza A can also undergo antigenic shift, whereby a strain with a new HA subtype enters and transmits readily in an immunologically naïve population. Antigenic shift is possible through reassortment resulting from the exchange of gene segments between two or more strains.

Reassortment plays a significant role in the evolution of Influenza A in the natural reservoir and during the emergence of pandemic strains. Because the variations of seasonal influenza viruses and pandemics can be unpredictable, current vaccines may not provide effective protection against them.

Universal vaccine can potentially provide broad protection against various types of influenza virus, so even if predictions are wrong, the vaccine will still be effective. Universal influenza vaccine approaches attempt to overcome the drawbacks of the highly changing nature of influenza viruses. The objective of universal vaccines is to induce cross-protective broadly neutralizing immunity, which depends on stimulating both humoral and cell-mediated arms of the immune system. Universal vaccines rely on the concept of developing immune responses against conserved viral epitopes. To develop universal influenza vaccines, conserved sequences that are shared by different influenza viruses must be used as vaccine antigens. M2 of influenza virus matrix protein has remained fairly conserved since the 1918 influenza outbreak, and thus M2 is an attractive target to develop a universal influenza vaccine.

However, M2 is the protein in very small numbers on the virus surface and is poorly immunogenic. Various approaches have been employed including fusion of M2 to different carriers such as hepatitis B virus core protein, bacterially-derived outer membrane vesicles in order to enhance the immunogenicity of M2 [11],[12]. Each construct can display M2 on HBc particle or *E.coli*, respectively. M2 vaccine candidates have been successfully tested for efficacy against a panel of divergent influenza viruses in animal models. Clinical studies have been conducted with M2 vaccine candidates, which demonstrated their safety and immunogenicity in humans. [13].

Many infectious agents enter the body through mucosal surfaces. Mucosal immunity is being recognized to be important for providing effective protection against pathogens entering mucosal surfaces [14]. Influenza is a respiratory disease by influenza virus that enters through mucosal surface. Mucosal immunity acts as the front line of host defense by blocking influenza virus from infecting the upper respiratory tract and spreading to the lower respiratory trac. In the mucosal immunity IgA plays an important role. Therefore, efficacious influenza vaccine should induce IgA in the respiratory tract. In order to induce IgA in the mucosa, it is necessary to administer viral antigens via the mucosa with appropriate adjuvants.

Intranasal vaccination is an attractive route as a needle-free vaccine delivery method. When inactivated influenza vaccines were administered intranasally with an appropriate adjuvant, IgA was induced in the respiratory tract [15]. Several preclinical studies on adjuvant-combined, nasal-inactivated vaccines showed protection against infection of influenza virus [16]. Furthermore, studies on influenza vaccine combining universal vaccine and mucosal administration have been conducted. Intranasal vaccination with VLP displaying M2 could induce humoral and cellular immune responses conferring cross-protection against heterosubtypic influenza viruses [17].

III. PERTUSSIS VACCINE

Pertussis is a severe respiratory disease in infants, young children and even in adults [18]-[20]. It is classically known as pertussis because of its characteristic cough. It is most caused by the bacterium *Bordetella pertussis* (*B. pertussis*). The typical presentation of pertussis is seen in unimmunized children (less frequently in adolescents and adults) and is a three-stage illness: catarrhal, paroxysmal, and convalescent.

Pertussis was responsible for high mortality rates before the introduction of effective vaccines in the second half of the 20th century. Pertussis is the prevalent vaccine-preventable disease in the developed world. The first vaccine against pertussis was the whole-cell vaccine. However, it was replaced by the acellular vaccine because the whole-cell vaccine caused side effects. Currently, the preferred vaccine in most industrialized nations is the acellular vaccine [21],[22]. The acellular vaccine is created using antigen proteins identified and isolated from a pathogen. In order for the acellular vaccine to be effective, it must provide an immune response similar to that triggered by direct contact with the pathogen itself.

The earliest form of this vaccine was created in 1981 by a Japanese researcher. The current acellular vaccine is made up of 5 different antigens: PT, FHA, PRN, and fimbriae. Animal models and human clinical trials were used to assess which virulence factors produce protective antigens upon exposure. The vaccine differs significantly from one manufacturer to the next, as there are no standardized strains from which these antigens are purified from, and no standardization for the processes of purification, detoxification, or incorporation of adjuvants and preservatives.

The components of the acellular vaccine are combined with diphtheria and tetanus toxoids (DTaP). This vaccine is usually administered 5 times during childhood and once more during early adolescence. In order to combat the increased strength and consequently reaction of an older immune system, the “boosters” after the original inoculation contain lower concentrations of the antigens, in order to minimize their reactogenicity. The DTaP vaccine has been successful because it effectively minimizes the adverse side effects so prevalent in the DTwP vaccine. Despite the widespread use of the acellular vaccine, pertussis has recently been on the rise.

Natural infection with *B. pertussis* has long been considered to induce strong and long-lasting immunity that wanes later than vaccine-induced immunity. Therefore, live vaccines could be possible to mimic as closely as natural infection. Mielcarek et al. developed such a live vaccine candidate [23]-[25]. They constructed attenuated live vaccine BPZE1 by removing or altering genes that are involved in the pathogenesis of pertussis. Three virulence factors were genetically targeted:tracheal cytotoxin, pertussis toxin, and dermonecrotic toxin.

BPZE1 was found to induce protection in infant mice after a single intranasal administration that is superior to the protection provided by the current acellular vaccine. BPZE1 has completed a first-in-man phase I trial and was shown to be safe in young male volunteers, able to transiently colonize the nasopharynx and to induce antibody responses to *B. pertussis*

antigens [28]. Advantages of the use of BPZE1 include the relatively low production costs, making it especially attractive for developing countries, its needle-free, easy, and safe mode of administration, and its potential to induce mucosal immunity in addition to systemic immunity. This vaccine candidate may therefore be useful for long-term control of pertussis.

B. pertussis enters and colonizes the body through the respiratory mucosa which is the first line of the host defense against this pathogenic organism. Several studies have suggested the importance of local secretory antibodies (IgA) and Th1-type immune responses to protect B. pertussis [26]-[28]. Mucosally-administered vaccines can induce both the systemic and the mucosal immune responses while parenteral vaccines mainly activate the systemic immune response. Due to the fact that mucosally-administered soluble antigens are in general poorly immunogenic, several approaches, such as their encapsulation or expression in attenuated bacterial hosts, have been used to improve their immunogenicity. However, the attenuated bacterial hosts may still be able to cause a limited infection in infants as well as the aged and immunocompromised people. An attractive alternative to overcome this problem is the development of a new vaccine in association with lactic acid bacteria which are safe mucosal delivery vehicles [29].

In order to develop a simplified, cost-effective and well-defined vaccine candidate against *B. pertussis*, Torkashvand et al. constructed a food-grade expression system harboring a F1S1 fusion protein of B. pertussis to be produced in *Lactococcus lactis* (*L. lactis*) NZ3900 as a new oral vaccine model against pertussis, caused by *B. pertussis* [30]. F1S1 was composed of N-terminally truncated S1 subunit of pertussis toxin and type I immunodominant domain of filamentous hemagglutinin which are both known as protective immunogens against pertussis.

The recombinant *L. lactis* was administered via oral or intranasal routes to BALB/c mice and the related specific systemic and mucosal immune responses were then evaluated. The results indicated significantly higher levels of specific IgA in the lung extracts and IgG in sera of mucosally-immunized mice, compared to their controls. It was revealed that higher levels of IgG2a, compared to IgG1, were produced in all mucosally-immunized mice. Moreover, immunized mice developed Th1 responses with high levels of IFN-γ production by the spleen cells. These findings provide evidence for *L. lactis* to be used as a suitable vehicle for expression and delivery of F1S1 fusion protein to mucosa and induction of appropriate systemic and mucosal immune responses against pertussis.

IV. Vaccine Candidates for AD

AD is one of dementia, beginning at the age of 40 to 50 years, and one in two people is said to develop this disease at the age of 80 and over. One of the major histopathological characteristics of AD is the presence of senile plaques, composed mainly of amyloid β peptide (Aβ) aggregates [31],[32]. Aβ consists of 40 to 42 amino acids. Aβ forms soluble oligomers and the toxicity of oligomers to nerve cells is quite strong among them, so the Aβ oligomer has been considered to be a major cause of AD. Therefore, inhibition or elimination of formation of this soluble Aβ oligomer has been thought to lead to the prevention and treatment of AD.

The possibility of an AD vaccine was first demonstrated by immunization experiments using Aβ and model mouse of AD [33]. They showed that Aβ (1-42 aa) and a strong adjuvant QS21 were inoculated to transgenic mice PDAPP in which brain senile plaques are formed by high human APP expression. As a result, it was found that amyloid plaque formation and memory behavior disorder were prevented at the early stage of administration (6 weeks of age), and reduction of senile plaque and amelioration of memory behavior disorder were observed at the later time of administration (11 weeks of age). This study indicated that immunization with Aβ showed the possibility of being effective for prevention and treatment of AD.

Based on these results, clinical trials were conducted, and there was no safety problem with phase I. However, the clinical study was discontinued due to the occurrence of meningoencephalitis in 6% of patients at the stage of phase II [34]. The side effect is considered to be due to autoimmunity by T cells. Though the first clinical trial was discontinued due to meningoencephalitis, antibodies against Aβ was observed in patients receiving Aβ with adjuvant and senile plaque progression was also suppressed. It seemed likely that the Aβ vaccine might have worked prophylactically and therapeutically in patients with AD.

In response to this result, researches on Aβ vaccines that suppress cellular immunity have been processed so far. One approach is to delete T cell epitopes from Aβ and to enhance immunogenicity of the shorten peptides. Petrushina et al. designed the vaccine (PADRE-Aβ) consist of B cell epitope (Aβ 1-15 aa) in tandem and the universal T cell HLA DR epitope (PADRE) [35]. Immunization of BALB/c mice with the PADRE-Aβ produced high titers of anti- Aβ antibodies. Splenocytes from immunized mice showed robust T cell stimulation in response to peptides containing PADRE. On the other hand, splenocytes from immunized mice were not reactivated by the Aβ peptide. Their data suggest that PADRE-Aβ could bias the immune response toward a Th2 phenotype and/or replace the Aβ T cell epitope with a foreign T cell epitope and might prevent the adverse events that occurred during the first clinical trial.

Ghochikyan et al. compared the induction of humoral immune responses with Quil A (Th1-type adjuvant) and aluminium salt (Th2-type adjuvant) adjuvants singly and in combination, using PADRE-Aβ [36]. Their data indicated that the combined use of both Th2-type adjuvant and Th1-type adjuvant enhanced the therapeutically relevant anti-Aβ antibody production without inducing the potentially harmful Th1 immune response. Though alum is a Th2-type adjuvant, it is much less potent than the majority of Th1-type adjuvants including saponin (QS21). Therefore, they suggested that the combined use of both Th1- and Th2-type adjuvants could be more effective and safe vaccines for AD.

Matsuda et al. reported a simple technique of raising the immunogenicity of Aβ [37]. They conducted the study on Aβ

vaccine without adjuvant. According to their report, cysteine-binding Aβ could drastically induce antibody against Aβ without using adjuvants. Only binding cysteine to C-terminal of Aβ increased the immunogenicity. This cysteine binding effect was also observed with shorten Aβ peptides. The cysteine-binding Aβ could reduce accumulation of Aβ by administration to AD transgenic mice. It is noteworthy that accumulation of soluble Aβ, which is considered to be the cause of AD, could also be prevented. This simple technique would provide Inexpensive and safe AD vaccine.

Wiessner et al. designed CAD106 which is the virus-like particle (VLP) conjugated with Aβ. In order to avoid activating Aβ-specific T-cells, they chose the Aβ1–6 peptide (DAEFRH) as antigen, which is shorter than typical T-cell epitopes and resides outside the region of Aβ reacting with T-cells. It was extended by a spacer (GGC) and covalently conjugated to the VLP derived from Escherichia coli RNA phage Qβ [38]. Each VLP contains 350–550 Aβ peptides. Immunization with CAD106 did not activate Aβ-specific T-cells. In AD transgenic mice, CAD106 induced efficacious Aβ antibody titers of different IgG subclasses mainly recognizing the Aβ3-6 epitope. CAD106 reduced brain amyloid accumulation in two AD transgenic mouse lines. In rhesus monkeys, CAD106 induced a similar antibody response as in mice. The antibodies stained amyloid deposits on tissue sections of mouse and human brain but did not label cellular structures containing APP. The antibodies reacted with Aβ monomers and oligomers and blocked Aβ toxicity in cell culture. CAD106 first-in-human study demonstrated a favorable safety profile and promising antibody response [39].

Fu et al. reported bacterium-like particle (BLP) carrying Aβ1-6 for AD vaccine. Different copy numbers of the Aβ1-6 peptide were specifically loaded on the surface of BLPs via fusion with a peptidoglycan anchoring domain [40]. These four BLP-based Aβ vaccines successfully induced high levels of Aβ42-specific antibodies in mice. However, none of the vaccines induced a T-cell-mediated immune response. Among the four vaccines, 6 copy-Aβ1-6-PA-BLP was the most effective in inducing Aβ-specific antibodies, indicating that a suitable epitope copy number is critical for high immunogenicity of the BLP-based vaccine. Furthermore, high levels of serum Aβ-specific antibodies could still be detected 3 months after the final administration of 6 copy-Aβ1-6 -PA-BLP.

V. CONCLUSION

The majority of currently licensed influenza vaccines are influenza HA component vaccines, whicht are administered by intramuscular injection. Those current influenza vaccines cannot response to virus mutants and cannot protect invasion of the virus from mucosa. In order to overcome the drawbacks, it is necessary for new vaccines to have features covering viral mutations and preventing viral invasion from upper respiratory mucosa. Universal influenza vaccine with conserved region among influenza viruses and mucosal influenza vaccines inducing IgA in mucosa would be promising candidates of new vaccines.

The first vaccine against pertussis was the whole-cell vaccine. However, it was replaced by the acellular vaccine because the whole-cell vaccine caused side effects. Though the acellular vaccine has been widely used in the world, pertussis has been not eradicated and tends to increase gradually. In order to overcome such situations, new vaccines have been developed. One of them is a genetically modified live vaccine. In addition, a mucosal vaccine using lactic acid bacteria carrying components of pertussis and a bacterium-like particle vaccine with components of pertussis are developing. These new vaccines would be expected to control pertussis.

With the aging of the population, patients with AD has increased. Aβ is considered as a cause of AD. Since the vaccine against Aβ has been shown to become an AD vaccine, a number of studies have been conducted worldwide. AD is a serious non-infectious disease. The development of the AD vaccine will also accelerate the development of vaccines for other non-infectious diseases like high blood pressure, diabetes and Parkinson's disease.

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