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Dendritic cell-targeting DNA-based mucosal adjuvants for the development of mucosal vaccines

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Abstract

In order to establish effective mucosal immunity against various mucosal pathogens, vaccines must be delivered via the mucosal route and contain effective adjuvant(s). Since mucosal adjuvants can simply mix with the antigen, it is relatively easy to adapt them for different types of vaccine development. Even in simple admixture vaccines, the adjuvant itself must be prepared without any complications. Thus, CpG oligodeoxynucleotides or plasmids encoding certain cDNA(s) would be potent mucosal adjuvant candidates when compared with other substances that can be used as mucosal adjuvants. The strategy of a DNA-based mucosal adjuvant facilitates the targeting of mucosal dendritic cells, and thus is an effective and safe approach. It would also provide great flexibility for the development of effective vaccines for various mucosal pathogens.

Keywords

CpG ODN; Flt3 ligand; mucosa; secretory IgA; vaccine

Mucosal immune system

The mucosal immune system consists of an integrated network of tissues, lymphoid and mucous membrane-associated cells, and effector innate (e.g., mucins and defensins) and acquired (e.g., antibodies [Abs]) molecules. Along with cytokines, chemokines and their receptors, these effector Ab molecules, which are primarily of the IgA isotype, are key players in mucosal immunity and appear to function in synergy with the innate immune system operated by Toll-like receptors (TLRs) [1,2]. Mucosal inductive sites include Peyer's patches (PPs), one of the well-characterized gut-associated lymphoreticular tissues and Waldeyer's ring of tonsils and adenoids as nasopharyngeal-associated lymphoreticular tissues (NALT). These tissues collectively comprise a mucosa-associated lymphoid tissue (MALT) network, which continuously supplies antigen (Ag)-specific cells and memory B and T cells to diffuse mucosal effector sites where IgA Abs are continuously converted into the secretory form of IgA (S-IgA) [1,2]. The migration of lymphocytes from inductive to mucosal effector tissues is the cellular basis for the originally described concept of the common mucosal immune

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system, where either nasal or oral vaccination induces mucosal immunity in distant multiple effector sites [1,2].

The MALTs (including PPs) are covered by a lymphoepithelium containing microfold (M) cells and well-organized regions, the subepithelium dome (SED) containing antigen-presenting cells (APCs) or dendritic cells (DCs), a B-cell zone with germinal centers, and adjacent T-cell areas with APCs, including follicular DCs and high endothelial venules. Naive, recirculating B and T lymphocytes enter MALT via the high endothelial venules [1,2]. Covering the MALT is a follicle-associated epithelium that contains a subset of differentiated epithelial cells termed M cells, as well as columnar epithelial cells and lymphoid cells [2]. The follicle-associated epithelium M cell plays a crucial role in the initial phase of induction of mucosal immune responses by sampling Ags from the lumen of the gut or nasal passages and transporting the intact form of Ags to the underlying APCs for initiation of the Ag-specific immune response. Furthermore, Ag-activated and memory B- and T-cell populations then emigrate from the mucosal inductive environment via lymphatic drainage, circulate through the bloodstream and home to mucosal effector sites such as the lamina propria regions of the intestine and nasal passages (NPs), and glandular tissues. Resident in these mucosal effector sites, which are characterized by more diffuse and connective tissues, are the Ag-specific CD4⁺ Th1 cells and CD8⁺ cytotoxic T lymphocytes (CTLs) responsible for cell-mediated immunity (CMI)/CTL functions, as well as CD4⁺ Th2 cells and IgA-committed B lymphocytes responsible for IgA Ab responses [1,2]. Furthermore, recent evidence has indicated that regulatory T (Treg) cells and Th17 cells, involved in the suppression and protection or inflammation phases of the gut immune system, respectively, have been identified in the intestinal lamina propria (iLP) region [3–6].

In addition to this classical pathway to induce mucosal immune responses, recent findings show novel new mechanisms involved in the induction of the S-IgA Ab response. To this end, it was reported that DCs induced CD40-independent Ig class-switching [7–9]. Thus, DCs directly interact with B cells through the B-cell activation factor of the TNF family, also called lymphocyte-stimulator protein, and a proliferation-inducing ligand in order to induce surface IgA-positive (sIgA⁺) B cells (or post-switched IgA-committed B cells) [9]. Among these ligand–receptor interactions, proliferation-inducing ligand–transmembrane activator and CAML interactor (TACI) signal transduction plays a key role in the induction of CD40-independent IgA class-switching.

AB₅ toxin-based typical mucosal adjuvants

Mucosal adjuvants, live-attenuated microbial or particulate (including nanoparticles) delivery systems are required for effective mucosal immune responses (Figure 1) [1,2,10,11]. Adjuvants offer the advantage of eliciting mucosal as well as parenteral immune responses. One of the main advantages of employing the adjuvant is that vaccines can be simply prepared by admixture with the Ags. Thus, effective mucosal adjuvants would be useful tools for the development of mucosal vaccines for new and different pathogens. For example, it is relatively easy to adapt vaccines against the different types of seasonal influenza occurring each year. Two bacterial enterotoxins (native cholera toxin [nCT] and native human heat labile toxin type 1 [nLTh-1]) and their nontoxic mutants are well-established mucosal adjuvants for the induction of both mucosal and systemic immunity to coadministered protein Ags (Figure 1) [1,2,12–18]. Mucosal administration of vaccine together with nCT or nontoxic mutant CTs (mCTs) tends to induce CD4⁺ Th2-type cells with characteristic plasma IgG1, IgG2b, IgE and IgA, as well as mucosal S-IgA Ab responses, whereas nLTh-1 preferentially induced both Th1- and Th2-type cytokine responses with systemic IgG1 and IgG2a, and mucosal S-IgA Ab responses [12,19]. Since both enterotoxins possess severe toxicities and are thus unsuitable for use in humans, several studies have investigated the potential adjuvant effect of nontoxic

derivatives of CT and heat-labile toxin (LT) that lack toxicity but retain adjuvanticity [20–24]. In particular, single amino acid substitution mutants in the A subunit of LTs (R7K, S63K and R192G) and CTs (S61F and E112K), which lack ADP-ribosyltransferase activity, were shown to retain their adjuvant properties [13,14,25,26]. Mucosal adjuvant activity of mCTs S61F and E112K have been shown using several Ags including ovalbumin (OVA), diphtheria and tetanus toxoid (TT), and influenza virus [17]. Moreover, an additional mutation was added to mCT E112K in order to prevent intracellular trafficking of the CT-A subunit. Thus, double-mutant CTs showed significant adjuvant properties with minimum toxicity when compared with the single mCT E112K [27]. These findings provide direct evidence that neither the ADP ribosyltransferase activity nor normal intracellular trafficking of CTs were required for its mucosal adjuvant activity. In addition to enterotoxin-based typical mucosal adjuvant, cytokines, chemokines, growth factors, chemicals (e.g., QS21), TLR ligands and edible plants possess potent mucosal adjuvanticity for vaccine development. However, in this review, we focus our discussion on mucosal DC-targeting DNA-based adjuvant and its current preferred immunization route.

Mucosal cytokines & innate factors as adjuvants

Mucosal delivery of cytokines allows the use of these mediators of the immune response without the adverse effects associated with the large and repeated parenteral doses generally required for the effective targeting of tissues and organs (Figure 1). For example, following nasal delivery of IL-12, significant serum levels equivalent to approximately one-tenth of those achieved by parenteral injection were achieved [28]. Moreover, serum IFN- γ levels were ten-times lower, confirming the biological activity of the nasally administered cytokine [16]. A nasal vaccine of tetanus toxoid (TT), administered with either IL-6 or IL-12, induced serum TT-specific IgG Ab responses that protected mice against lethal challenge with TTs, suggesting that both IL-6 and IL-12 can enhance protective systemic immunity to mucosal vaccines [29]. Furthermore, nasal administration of TTs with IL-12 as adjuvant induced high titers of S-IgA Ab responses in the GI tract, vaginal washes and saliva [29]. Similar results were reported when mice were nasally immunized with influenza H1N1 protein or pneumococcal polysaccharide conjugate vaccines together with IL-12 [30,31], demonstrating that nasal IL-12 does not require additional stimuli for the induction of S-IgA Ab responses. In related studies, IL-12 was shown to redirect CT-induced antigen-specific Th2-type responses toward the Th1 type when administered via oral [32] or nasal routes [28]. IL-12 was also shown to promote both Th1- and Th2-type responses when administered by a separate mucosal route [28]. These observations clearly show that IL-12 is also a powerful regulatory cytokine for the induction of targeted immunity.

A number of studies have addressed whether innate molecules secreted in the epithelium could provide signals to bridge the innate and adaptive mucosal immune systems (Figure 1). To test this concept, protein antigens were administered with either IL-1 [33], α -defensins [34] or lymphotactin [35]. Lymphotactin, a C chemokine produced by natural killer (NK) and CD8 T cells such as $\gamma\delta$ TCR⁺ intraepithelial lymphocytes, is chemotactic for T and NK cells, and induces the migration of memory T cells across endothelial cells [36]. IL-1 is produced by a number of cells, including macrophages and epithelial cells, whereas α -defensins are produced by Paneth cells. Nasal administration of protein antigens with these innate molecules enhanced systemic immune responses to coadministered antigens [33–35]. However, whereas both IL-1 and lymphotactin produced mucosal S-IgA Ab responses, the defensins failed to do so [33–35]. These studies show that inflammatory cytokines and molecules of the innate immune system can be effectively administered by mucosal routes to regulate both systemic and mucosal immune responses.

Mucosal DCs

Immunohistological analysis of murine PPs has shown that CD11c⁺, CD11b⁺, CD8⁻ immature DCs with high endocytic activity, and low levels of MHC and B7 molecule expression form a dense layer of cells in the SED [37]. These CD11c⁺, CD11b⁺, CD8⁻ immature DCs are of the myeloid type and express C-C chemokine receptor (CCR)6 for directing their migration towards the SED [38]. Furthermore, myeloid-type CD11b⁺ DCs are known to migrate into the T-cell zone after Ag uptake and to begin the process of maturation by expressing CCR7 [38]. Mature interdigitating CD11c⁺, CD11b⁻, CD8⁺ DCs with low endocytic activity and high numbers of MHC class I and class II, as well as B7, molecules have been identified in the interfollicular T-cell regions [37], and are known to express high levels of CCR7 [38]. It is known that PP DCs preferentially promote CD4⁺ Th2-type responses [38], suggesting that these DCs also influence the nature of immune responses initiated in the GI tract. DCs are also found in the NALT, where they appear to play the same role as those seen in the PPs. However, it should be noted that little is currently known about the nature and immunological characteristics of DC subsets residing in the NALT. Finally, DCs are also found in human tonsils, a mucosal inductive site [39]. They are recruited into the respiratory epithelium during acute immune responses induced by bacteria, viruses or protein Ags [40]. Although freshly isolated rat respiratory DCs preferentially support CD4⁺ Th2-type responses, induction of Th1-type cytokine-mediated immune responses was also seen after stimulation with OVA [41].

Owing to their structural resemblance to Ig-secreting plasma cells, one subset of DCs in humans was recently identified as plasmacytoid DCs (pDCs) [42]. Possessed of a unique surface phenotype (CD4⁺, IL-3R^{high}, CD45RA⁺, HLA-DR⁺) [43], pDCs can produce high levels of IFN- α in response to viral stimulation [44,45] or to oligodeoxynucleotides (ODNs) containing particular cytidine phosphoguanosine (CpG) motifs [46]. Human pDCs show less phagocytic activity, lower IL-12 responses and fewer activation-inducing signals than do conventional myeloid DCs [43,46–51]. Like human pDCs, murine pDCs express CD11c^{low}, B220^{high}, Gr-1^{low}, show a plasmacytoid morphology and produce IFN- α in response to viral stimulation [52–54]. In the presence of Flt3 ligand (FL), they can be differentiated in high numbers from bone marrow cells [55–57]. A recent study showed that PPs contain pDCs, although these are of a distinctive CD8⁺, CD11c⁺ phenotype not seen in other lymphoid tissues [58].

It is well known that DCs play a key role in immune surveillance and possess unique functions in the stimulation of naive T cells to differentiate into either Th1- or Th2-type cell responses. A variety of molecules, such as bacterial-derived Ags, viral products, growth factors, cytokines and chemokines, can promote DC activation, expansion and maturation [59,60]. It has been shown that mucosal DCs play additional distinct roles in the regulation and induction of S-IgA Ab responses. Vitamin A deficiency with impaired mucosal IgA Ab responses is a well-known phenomenon [61,62]; however, the precise mechanisms involved remained unknown until recently. A series of elegant studies showed that retinoic acid (RA)-producing mucosal DCs play key roles in the regulation of mucosal homeostasis and immunity. In this regard, RA-producing DCs in PPs preferentially upregulate receptors for intestinal tropism on T and B cells for the induction of mucosal S-IgA Ab responses at distant lamina propria regions of the intestine [63–65]. In addition, it was also reported that the RA-producing CD11b⁻ DC subset in the iLP is known to induce Treg cells in the presence of TGF- β ₁, whereas CD11b⁺ DCs in the iLP preferentially promote Th17-cell differentiation, as well as B-cell IgA class-switching and subsequently IgA synthesis [66–70]. These findings emphasize that appropriate DC targeting by mucosal vaccines is the key issue for the induction of effective mucosal immune responses.

DNA-based molecular adjuvants that target DCs

CpG ODN

It has been shown that bacterial DNA and pathogen-associated molecular patterns contain a significantly high frequency of unmethylated CpG motifs [71,72]. The unmethylated CpG motifs are recognized by the innate immune system via TLR9, which is expressed by B cells and pDCs [73]. Thus, CpG DNA induces the maturation and stimulation of professional pDCs, as well as subsequent Ag-specific Th1 and CTL responses [74,75]. Synthetic CpG ODNs induced protective immune responses similar to those triggered by bacterial DNA [76–79]. Since CpG ODNs are potent immunomodulators capable of targeting malignant tumors and diminishing allergic responses, CpG motifs are thought to be of central importance in TLR9-mediated innate immunity [80,81]. In addition, CpG ODNs act as effective adjuvants for the induction of Ag-specific immunity [82]. Indeed, CpG ODNs enhanced both Ab and CMI responses to OVA in mice [83]. Furthermore, when viral or toxoid vaccines were administered with CpG ODN, significantly increased levels of Ag-specific Ab and CTL responses were seen [84–89]. The mucosal delivery of CpG ODN plus formalin-inactivated influenza virus or HBV surface antigen successfully induced Ag-specific Ab responses in both the external secretions and plasma of mice [88,89]. It is also an effective mucosal adjuvant for both hepatitis B Ags and TTs administered orally [90]. This CpG DNA adjuvant is so potent that it may even change a predominant Th2- into a Th1-type response [91]. The precise mechanisms for CpG DNA adjuvanticity are not yet known; however, CpG DNA upregulates mitogen-activated protein kinases associated with IL-12 production by APCs [92]. Our recent studies showed that mice given a nasal recombinant protective antigen (PA) of the anthrax lethal toxin plus CpG ODN exhibited high levels of PA-specific IgG2a and IgA Ab responses in both plasma and external secretions [93]. Importantly, these PA-specific Abs neutralized the lethal toxin *in vitro* [93].

Plasmid-encoding cytokine or growth factor

The mucosal administration of plasmid-encoding cDNA of cytokines, chemokines or growth factors and expression promoters may be the convenient approach to effectively deliver these factors to mucosal tissues. Indeed, it has been shown that nasal application of the TGF- β_1 plasmid effectively prevents the induction of experimental colitis [94]. Thus, several groups, including ours, have adapted the cDNA plasmid as a mucosal adjuvant [95–99]. As described previously, since IL-12 showed significant mucosal adjuvanticity, nasal application of plasmid-encoding IL-12 cDNA was employed for the prevention and treatment of intestinal allergic diarrhea [96]. Furthermore, recent studies showed that the IL-12 cDNA vaccine coexpressing *Yersinia pestis* F1-V fusion protein confers protection against pneumonic plague [99]. These studies clearly suggest that the IL-12 cDNA plasmid is a potent mucosal adjuvant to induce protective immunity against pathogens. Others showed that IL-15 cDNA plasmid as systemic adjuvant enhanced Ag-specific proliferative responses and IFN- γ production by CD8⁺ T cells [100]. In addition, coimmunization with plasmid-expressing influenza hemagglutinin, together with IL-15 plasmid, induced CD8⁺ T-cell immunity and protected the mice against lethal mucosal challenge with influenza virus [100]. Recent studies also reported that coadministration of IL-15 cDNA plasmid as nasal adjuvant enhanced cell-mediated immunity when compared with those responses induced by Ag alone [98]. Thus, high levels of T-cell proliferative and CTL responses, as well as increased levels of IFN- γ production by both CD4⁺ and CD8⁺ T cells, were observed in mice given IL-15 plasmid as a nasal adjuvant [98]. As mentioned previously, cytokines and chemokines show significant mucosal adjuvanticity and therefore might be good candidates for plasmid-based adjuvants.

The Flt3 ligand, which binds to the *fms*-like tyrosine kinase receptor Flt3/Flk2, is a growth factor that dramatically increases the numbers of DCs *in vivo* without inducing their activation [101,102]. Treatment of mice by systemic FL injection induced marked increases in the

numbers of DCs in both systemic (i.e., spleen) and mucosal lymphoid tissues (i.e., iLP, PPs and mesenteric lymph nodes) [103], thereby enhancing the induction of oral tolerance [104]. Other studies have now shown that FL treatment also favors the induction of immune responses after mucosal [104], systemic [105] or cutaneous [106] vaccine delivery. In recent studies, plasmid DNA encoding FL (pFL) has been coadministered with plasmids encoding protein Ags or linked to the Ag itself [107,108]. These studies confirm the adjuvant activity of FL for both Ab and CMI responses, and suggest that costly treatment with FL protein may now be replaced by injection with FL cDNA. Building upon these early studies, we have focused on the design of second-generation mucosal adjuvants that can target and activate specific subsets of mucosal immunity-associated cells. We have selected FL as a mucosal DC-targeted adjuvant to induce maximum Ag-specific protective mucosal immunity. Our previous studies have shown that nasal application of OVA plus pFL induced CD8⁺ DC-mediated OVA-specific Ab responses in both mucosal and systemic lymphoid tissues (Figure 2) [97]. Thus, nasal delivery of a pFL as mucosal adjuvant preferentially expanded CD8⁺ DCs and subsequently induced Ag-specific, mucosal immune responses mediated by IL-4-producing CD4⁺ T cells (Figure 2) [97]. These studies clearly showed the target tissues and cells where the initiation of FL adjuvant function occurred. Thus, the highest expression of the plasmid-specific, ampicillin-resistant gene was in NALT followed by the NPs. The FL protein levels were significantly increased in nasal washes when compared with those from mice given Ag alone or empty plasmid. Similarly, FL levels in plasma were also elevated. Since the spleen as well as other lymph nodes did not express the plasmid-specific gene, we postulate that high levels of FL in plasma were primarily due to transudation from the nasal mucosa. These results show that FL-encoded plasmid was mainly taken up by NALT and subsequently the FL protein was produced locally in these tissues, which resulted in the subsequent expansion and activation of DCs.

Combined DNA double adjuvant for mucosal immunity

The induction of both Th1- and Th2-type responses is the major goal for the development of mucosal vaccines, since these responses would provide protective immunity against viral and bacterial infections by maximizing Ag-specific Ab and CTL responses in an immunocompromized situation. Furthermore, balanced Th1 and Th2 cytokine responses would avoid the induction of exaggerated Th1 cytokine-mediated inflammatory or Th2-related allergic responses. Since CpG ODN induces Th1-type cytokine-mediated immunity [93], whereas pFL preferentially promotes Th2-type cytokine responses [97], it is important to study whether a combination of pFL and CpG ODN as DCs targeting mucosal adjuvants would elicit enhanced Ag-specific Ab responses with balanced Th1 and Th2 cytokine responses. A recent study indeed showed that nasal delivery of a combination of pFL and CpG ODN enhances coadministered Ag; for example, OVA-specific mucosal and systemic immune responses similar to those induced by a single nasal adjuvant regimen (either pFL or CpG ODN) (Figure 3) [95]. Of note, the levels of prolonged mucosal immunity induced by the combined adjuvant displayed significantly higher responses when compared with nasal administration with OVA, and either pFL or CpG ODN as individual adjuvants [95]. In addition, the combination of pFL and CpG ODN as nasal adjuvants induced both CD4⁺ Th1- and Th2-type cytokine-mediated immune responses (Figure 3) [95]. The induction of prolonged Ag-specific immune responses was associated with elevated numbers of activated pDCs and CD8⁺ DCs in NALT. Importantly, significant levels of mucosal and systemic Ab responses were elicited even in 2-year-old mice by nasal delivery of the combined adjuvant (Figure 3) [95].

Safety of mucosal adjuvants & delivery systems

While diarrhea is the primary limiting factor for the use of oral enterotoxins as adjuvants in humans [109,110], major safety concerns with mucosal adjuvants and delivery systems for nasal vaccination are also important since they may enter and/or target olfactory neurons and,

therefore, may bypass the blood–brain barrier and gain access to olfactory bulbs (OBs) and deeper structures in the brain parenchyma [27,111–116]. Studies with enterotoxin adjuvants and, more recently, with a recombinant adenovirus (rAde) vector, suggest that these adverse effects are in large part mediated by the ADP-ribosyltransferase activity and the nature of the cellular receptors targeted. Both nCTs and nLTh-1 bind to GM1 gangliosides on epithelial cells, and require endocytosis followed by transport across the epithelial cell to reach the basolateral membrane. GM1 gangliosides are also abundantly expressed by cells of the CNS, and their concentration on neuronal and microglial cells varies during the development of various cell types and different regions of the brain [117]. nCTs or CT-Bs, when administered nasally to mice, entered the olfactory nerve and epithelium and OBs by mechanisms that were selectively dependent upon GM1 gangliosides [113]. These studies have also shown that nCTs as adjuvants promote the uptake of nasally coadministered, unrelated proteins into the olfactory nerve and epithelium [113]. No side effects were reported following nasal application of CT-Bs to 12 IgA nephropathy patients [118]. However, the targeting of CNS tissues by nasally administered bacterial enterotoxins is clearly related to a higher incidence of Bell's palsy (facial paresis) among volunteers of a nasal vaccination trial given nLTh-1 as mucosal adjuvant [119]. These studies clearly showed that CNS toxicity is the major concern for the development of effective mucosal adjuvants for nasal vaccines. Although an oral vaccine may be more tolerant than a nasal route of vaccine delivery in terms of their toxicity, it requires a large quantity of vaccines with higher adjuvant activity, when compared with nasal adjuvants, in order to achieve a certain level of efficacy. To this end, other routes of mucosal delivery systems, such as rectal, vaginal and sublingual immunization, have been discussed. Among them, sublingual immunization recently showed significant efficacy with a small volume of vaccine and potent safety [120–123]. Since sublingual immunization also facilitates targeting DCs in addition to nasal immunization [124], DNA-based vaccines could be adapted for this new delivery route.

Expert commentary

Adjuvants are presumably an essential element for the development of effective mucosal vaccines that could induce protective immunity at mucosal and systemic compartments. Currently, most desired mucosal vaccines should prevent infectious pathogens invading mucosal surfaces, which causes high severity and mortality. Both influenza virus and the bacterial *Streptococcus pneumoniae* (the pneumococcus) are upper-respiratory tract pathogens and significant causes of morbidity, and can result in death. To this end, the development of nasal vaccines, including adjuvants, are important for the prevention of these infectious diseases, since the nasal route of vaccination could most effectively induce pathogen-specific S-IgA Ab responses in the upper respiratory tract, although other routes of mucosal immunizations are available. In this regard, we seriously consider the development of safe and effective nasal adjuvants. For example, although FluMist[®], a trivalent nasal vaccine consisting of type A (H1N1 and H3N2) and type B live-attenuated influenza virus, might be a potent nasal vaccine on the current market, it is only approved for use in healthy people 2–49 years of age who are not pregnant. Thus, additional nasal adjuvants for nasal influenza vaccines that are safe and effective for all generations need to be developed. In this regard, it would be possible that a strategy of appropriate mucosal-targeting adjuvants, such as DNA-based nasal adjuvants, could be part of the solution for the development of safe and effective mucosal vaccines.

Five-year view

The major concerns for developing mucosal vaccines are efficacy and safety. In addition to these two issues, we must consider the time of availability. Future global warming could introduce unexpected pathogens that have never been seen before into new areas and cause pandemic infectious diseases. To prevent this type of catastrophe, it is essential to create a

strategy for facilitating new vaccine development. Thus, development of ‘the almighty mucosal adjuvant’, which can be used as admixture for any type of pathogen, would be the ideal, since this approach may develop effective and safe vaccines in a relatively short period of time. DNA-based mucosal adjuvants, including CpG ODNs and/or cDNA plasmids, could be potent candidates for the almighty mucosal adjuvant. To step forward to fulfill this role, the safety of plasmid cDNA must be extensively studied for human use, especially for nasal application.

Key issues

- The mucosal immune system is essential for the prevention of infectious diseases.
- Mucosal immunization induces two layers of immune defense (mucosal and systemic).
- Mucosal adjuvants are essential for the induction of effective mucosal immunity.
- Admixture of adjuvant(s) is simple and effective for developing vaccines.
- Mucosal dendritic cell-targeting is an effective and safe strategy for the induction of antigen-specific immunity.
- DNA-based adjuvants are relatively easy to construct and are potent and effective when compared with protein-based mucosal adjuvants.
- A combination of DNA-based mucosal adjuvant may lead to prolonged mucosal immune responses.

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Figure 1. The last 5 years of publications of mucosal vaccines, adjuvants, and delivery systems by 2002 and 2009

In order to induce effective mucosal immunity, mucosal adjuvants or live-attenuated microbial delivery systems are required. Enterotoxin-based mucosal adjuvants are the most potent and well-established strategy for the induction of both mucosal and systemic immunity to coadministered protein antigens. However, studies in toxin-based mucosal adjuvants are decreasing and the efforts are now focusing more on alternative safe and effective vaccine development.

CpG: Cytidine phosphoguanosine; ISCOM: Immune stimulating complex; VLP: Virus-like particle.

Figure 2. Dendritic cell-targeting Flt3 ligand-encoded plasmid as mucosal adjuvant

Nasal administration of pFL as mucosal adjuvant preferentially expands the numbers of nasopharyngeal-associated lymphoreticular tissue DCs for the induction of coadministered antigen-specific immune responses.

CMI: Cell-mediated immunity; CpG-ODN: Cytidine phosphoguanosine oligodeoxynucleotide; DC: Dendritic cell; pDC: Plasmacytoid dendritic cell; pFL: Flt3 ligand-encoded plasmid; TLR: Toll-like receptor.

Figure 3. DNA-based double mucosal adjuvants induce antigen-specific secretory IgA antibody responses and cell-mediated immunity

Nasal application of Flt3 ligand-encoded plasmid and CpG oligodeoxynucleotide selectively target CD8⁺ dendritic cells (DCs) and plasmacytoid DCs (pDCs), respectively. Subsequently, CD8⁺ DCs induce Th2-type cytokine responses, whereas pDCs promote Th1-type cytokine production for the induction of antigen-specific secretory IgA antibody responses and cell-mediated immunity.

B: B cell; NALT: Nasopharyngeal-associated lymphoreticular tissue; T: T cell; TM: Transmembrane.