Modes of Action for Mucosal Vaccine Adjuvants

Taiki Aoshi

Abstract

Vaccine adjuvants induce innate immune responses and the addition of adjuvants to the vaccine helps to induce protective immunity in the host. Vaccines utilizing live attenuated or killed whole pathogens usually contain endogenous adjuvants, such as bacterial cell wall products and their genomic nucleic acids, which act as pathogen-associated molecular patterns and are sufficient to induce adaptive immune responses. However, purified protein- or antigen-based vaccines, including component or recombinant vaccines, usually lose these endogenous innate immune stimulators, so the addition of an exogenous adjuvant is essential for the success of these vaccine types. Although this adjuvant requirement is mostly the same for parental and mucosal vaccines, the development of mucosal vaccine adjuvants requires the specialized consideration of adapting the adjuvants to characteristic mucosal conditions. This review provides a brief overview of mucosa-associated immune response induction processes, such as antigen uptake and dendritic cell subset-dependent antigen presentation. It also highlights several mucosal vaccine adjuvants from recent reports, particularly focusing on their modes of action.

Keywords: adjuvant, dendritic cells, vaccine, mucosal adjuvant

Introduction

VACCINATION IS ONE of the most influential achievements in medical history (47). Global smallpox eradication highlights the significant impact of vaccination. The smallpox vaccine was a live attenuated vaccine, and many current vaccines, such as measles, mumps, and rubella, apply the same strategy of utilizing live attenuated organisms for vaccination. Although these live attenuated vaccines are very effective, their application is limited to only immune healthy people due to the potential for live vaccine-derived infection progression in an immunocompromised host. This is a serious disadvantage of live attenuated vaccines, as demonstrated by the recently approved, live attenuated seasonal influenza virus vaccine FluMist (7,9), which is only approved for use in individuals of 2–49 years of age, leaving the most vulnerable populations, such as infants and the elderly, ineligible for FluMist application.

Inactivated or killed vaccines, and particularly recombinant or component vaccines composed of highly purified pathogen-derived antigen, are applicable for use in broader populations because these vaccines are not infectious. However, their immunogenicity, especially in the case of recombinant vaccines, is usually weaker than that of live attenuated vaccines because they lack the endogenous innate immune

stimulators that are required for inducing adaptive immune responses to the antigen (26). As such, in many cases, the addition of an external adjuvant as an exogenous innate immune stimulator is necessary to compensate this limitation of the purified vaccine. As examples, MF59 and AS03 were introduced as vaccine adjuvants for the 2009 pandemic influenza vaccine (42,58). Importantly, AS03 adjuvanted influenza vaccine was associated with an abrupt increase of narcolepsy in Finland and Sweden, but not with MF59, and the underlying mechanisms are still not understood (42,44). Given the importance of the production of safe and effective vaccines, this external adjuvant requirement has generated an increased focus on the vaccine adjuvant development (10,46).

There has also been a notable focus on the mucosal route of vaccination in recent years (30,40,59,60). Many pathogenic infections occur at the mucosal surface, making this site the first line of defense. Therefore, inducing protective immune responses by administering vaccines directly at the mucosa is considered an ideal way. However, attempts to develop mucosal vaccines have not yet been successful (28,39). This is partly due to the lack of knowledge of how mucosal adjuvants work and of how to select appropriate (safe and effective) adjuvant candidates for the target disease. Although there are many similarities to the process of adjuvant development for

parental vaccines, the development of mucosal adjuvants requires some specialized considerations. This review provides an overview of vaccine adjuvants and the role of dendritic cell (DC) subsets, after which it highlights several mucosal vaccine adjuvants, especially nasal vaccine adjuvants from recent reports, focusing on their modes of action.

Vaccine Adjuvants and Their Classification

A variety of substances, including particles, chemicals, and oils, have been demonstrated to work as vaccine adjuvants in animal experiments and clinical trials (10,46). Their modes of action can be categorized into two major classes: innate immune receptor agonists and others (Fig. 1). Innate immune receptor agonists function as pathogen-associated molecular patterns (PAMPs). They are directly recognized by innate immune receptors, such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), Nod-like receptors (NLRs), C-type lectin receptors, AIM2-like receptors (ALRs), and cGAS/STING, and induce proinflammatory cytokine and interferon responses, collectively referred to as ''innate immune responses'' (5,54,61). The modes of action in the ''other'' category can be further subdivided into damage-associated molecular pattern (DAMP) inducers and pure antigen delivery systems. Adjuvants that are categorized as DAMP inducers do not cause any innate immune responses *in vitro* (such as cytokine secretion or DC maturation); they only induce innate

immune responses *in vivo*. These injected substances act by damaging the host cells, causing them to release several factors classified as DAMPs (e.g., DNA and RNA), which subsequently activate innate immune receptors (20,45). Notably, DAMP-inducer adjuvants are usually nonpathogen-derived substances, such as lipids, mineral salts, or polymer particles. In contrast, the pure delivery system adjuvants are totally inert both *in vitro* and *in vivo*, in terms of innate immune response induction. Instead, they very efficiently deliver the vaccine antigen to antigen-presenting cells, such as DCs, and the subsequent DC-mediated antigen presentation results in an enhanced adaptive immune response (Fig. 1).

Antigen Uptake Through Mucosal Surfaces

Mucosal surfaces, which by definition are covered by mucus, act as physical barriers preventing vaccine antigens and adjuvants from reaching the mucosal epithelial cells and other potential antigen transporter cells, such as goblet cells (36), transepithelial dendrite (TED)-forming CX3CR1[+] macrophages (35), M cells (25), and intraepithelial DCs (17,64) at the mucosa. The cells composing the mucosal epithelium are mutually interconnected by tight junctions and form an impermeable barrier to foreign substances (65). Small chemicals, such the c-di-GMP adjuvant (discussed below; molecular weight = ~690 g/mol), can be passively diffused and are able to cross this barrier through the
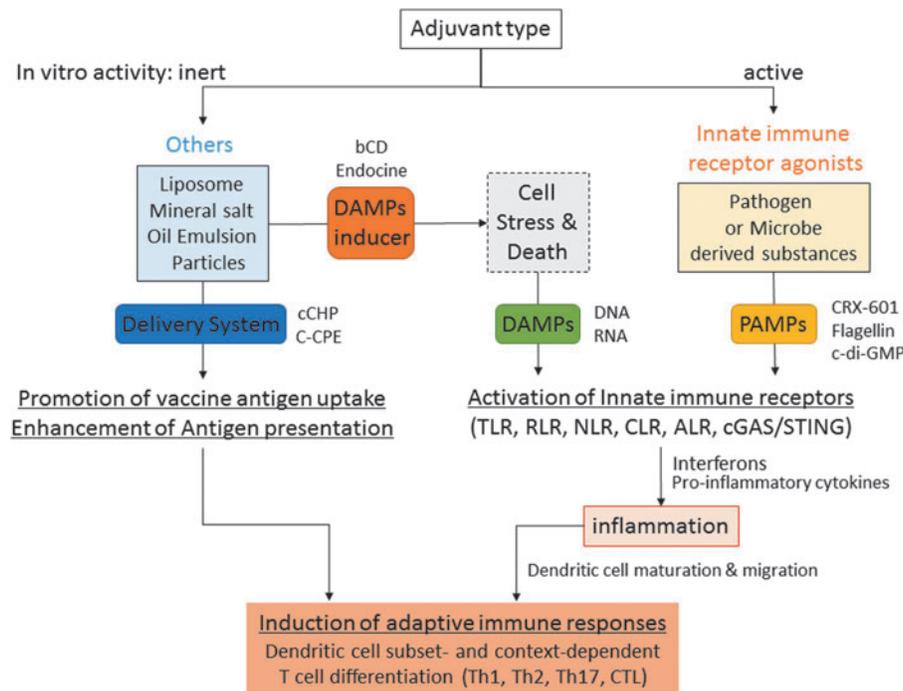

FIG. 1. Adjuvant classification chart based on the mode of action. The adjuvant can be divided into two types based on the presence or absence of *in vitro* innate immune stimulating activities. ''Innate immune receptor agonists'' are mostly pathogen- or microbe-derived substances, and work as PAMPs. ''Others'' mainly consist of nonpathogen-derived substances. ''Others'' are further divided into ''DAMP inducer'' and ''Delivery system.'' Both PAMP and DAMP adjuvants activate innate immune receptors and resulted in cytokine responses and dendritic cell maturation/migration. ''Delivery system'' promotes vaccine uptake and enhances antigen presentation by dendritic cells. ALR, AIM2-like receptor; bCD, hydroxypropyl-*β*-cyclodextrin; cCHP, cationic cholesteryl group-bearing pullulan; C-CPE, a C-terminal fragment of *Clostridium perfringens* enterotoxin; CLR, C-type lectin receptor; DAMP, damage-associated molecular pattern; NLR, Nod-like receptor; PAMP, pathogen-associated molecular pattern; RLR, RIG-I like receptor, TLR, Toll-like receptor.
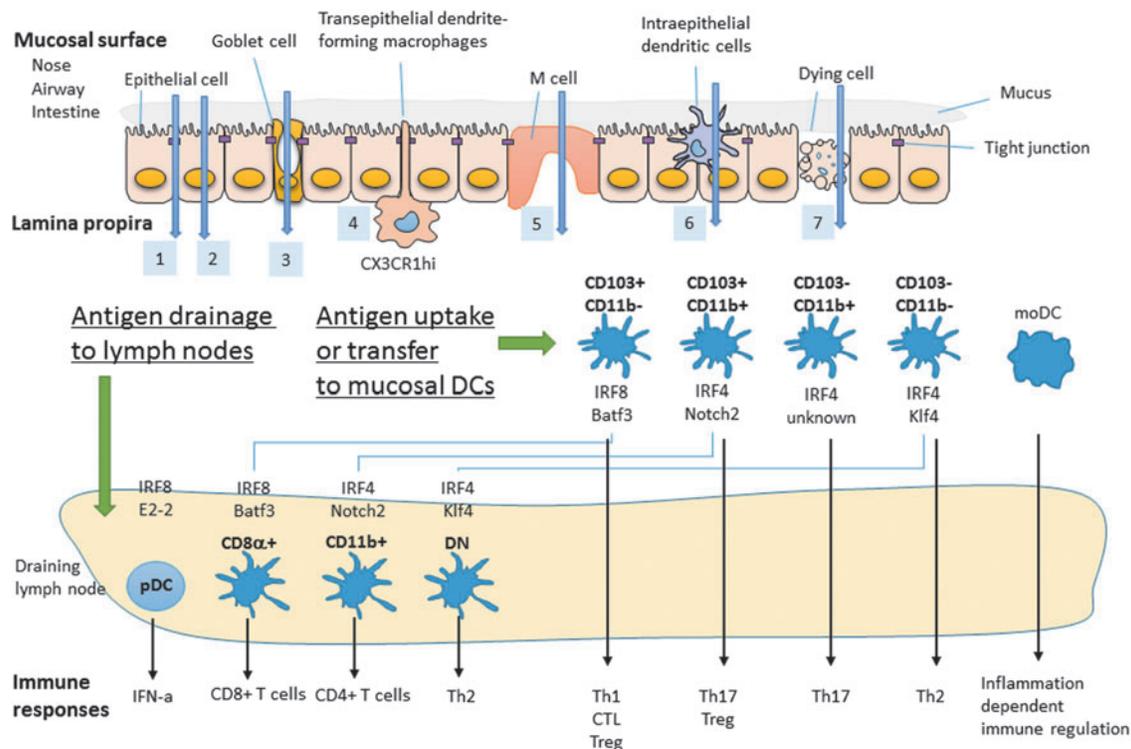
FIG. 2. Antigen transport across the mucosal barrier and DC subset-dependent immune responses. Antigen and adjuvant can cross the mucosal barrier through the following pathways: (1) paracellular pathway, (2) transepithelial pathway, (3) goblet pathway, (4) TED pathway, (5) M-cell pathway, (6) IED pathway, and (7) epithelial cell damage pathway. The translocated antigens and adjuvants are subsequently taken up by several different mucosal tissue DCs. These antigen-carrying DCs migrate to the draining lymph nodes for antigen presentation to T cells, where they induce characteristic T cell differentiation dependent on their specialized function and associated immune context. IED, intraepithelial dendritic cell; TED, transepithelial dendrite.

intercellular space between epithelial cells; this mechanism is called the paracellular pathway (Fig. 2, pathway 1). Epithelial cell-targeted antigens, such as FcRn- (63) or claudin 4- (53) targeted antigens (both discussed below), are transported by receptor-mediated transcytosis of epithelial cells in the transepithelial pathway (Fig. 2, pathway 2). Experimental soluble antigens, such as ovalbumin or dextran, can be taken up by goblet cells in the goblet pathway (Fig. 2, pathway 3) (36) or by TED-forming CX3CR1[+] macrophages in the TED pathway (Fig. 2, pathway 4) (35). Nanoparticles and some bacteria are taken up by M cells in the M-cell pathway (25) (Fig. 2, pathway 5) or by intraepithelial DCs in the intraepithelial DC (IED) pathway (17,64) (Fig. 2, pathway 6). Epithelial cell damage also physically breaks the mucosal barrier, allowing antigens to be transported into the lamina propria through the epithelial cell damage pathway (Fig. 2, pathway 7). For all pathways, the translocated antigens are taken up by mucosal tissue DCs, after which some of these antigen-carrying DCs migrate to the draining lymph nodes.

DC Subsets in Lymphoid and Mucosal Tissues

DCs are important cells that bridge innate and adaptive immune responses, and they have been shown to play a critical role in antigen presentation and tolerance induction. Recent extensive analyses of the cell surface makers and the critical growth and transcription factors involved in DC dif-

ferentiation have established that DCs form a heterogeneous cell population, and their comprehensive transcriptome data are also open for public use by the Immunological Genome Project (www.immgen.org). These DC subsets are functionally distinct and differentially affect T cell differentiation into Th1, Th2, Th17, CTL, and regulatory T (Treg) cells (21,37,38,50). Although most of the experiments defining these subsets were performed in mice, similar DC subsets have been shown to exist in humans (49). In steady-state lymphoid tissue, conventional DCs (cDCs) and plasmacytoid DCs (pDCs) are identified residentially. Lymphoid tissue-resident cDCs are further divided to three cDC subsets: CD8α^{+}/CD4^{−}/CD11b^{−}/CD24^{+}/SIRPα^{−} (referred to in this review as CD8α^{+}) DC, CD8α^{−}/CD4^{+}/CD11b^{+}/CD24^{−}/SIRPα^{+}/ESAM^{hi} (CD11b^{+}) DC, and CD8α^{−}/CD4^{−}/CD11b^{+}/CD24^{−}/SIRPα^{−}/ESAM^{lo} double-negative (DN) DC (2,37,38) (Fig. 2). In peripheral nonlymphoid tissue, such as the lung and intestinal lamina propria, four phenotypically different DC subsets can be distinguished, CD103^{+}/CD11b^{−} DC, CD103^{+}/CD11b^{+} DC, CD103^{−}/CD11b^{+} DC, and CD103^{−}/CD11b^{−} DC, but these occur in different population ratios depending on the examined organ (8,16,51) (Fig. 2, bottom). Analyses from several transcriptional factor-deficient mice revealed that pDC, CD8α^{+} DC, and CD103^{+}/CD11b^{−} DC are an IRF8-dependent population (38). In contrast, CD11b^{+} DC, CD103^{+}/CD11b^{+} DC, CD103^{−}/CD11b^{+} DC, and CD103^{−}/CD11b^{−} DC are an IRF4-dependent population (38,51,55). An analysis using Batf3-

deficient mice (24) showed that lymphoid tissue-resident CD8α⁺ DCs and peripheral tissue CD103⁺/CD11b⁻ DCs are related populations (16). Similarly, Notch2 is required for lymphoid tissue CD11b⁺ DCs and peripheral tissue CD103⁺/CD11b⁺ DC differentiation, suggesting that these DCs are related populations (29,48). Furthermore, Klf4 is required for DN DCs and CD103⁻/CD11b⁻ DCs (55), suggesting that these two DC subsets are also related through Klf4 dependency.

These lymphoid tissue-resident and peripheral non-lymphoid/migratory DCs have largely distinct, but some overlapping, functions. Seminal work by Dudziak *et al.* first demonstrated that lymphoid tissue-resident CD8α⁺ DCs and CD11b⁺ DCs are functionally different for preferential antigen presentation to CD8⁺ T cells and preferential antigen presentation to CD4⁺ T cells, respectively (15) (Fig. 2). This DC subset marker and the functional connection seem to also hold true for other lymphoid and nonlymphoid DC populations. CD103⁺/CD11b⁻ DCs play a critical role for Th1 cell (34) and CTL (14,16,19,24) induction and are also involved in some part of Treg induction (11). In addition, CD103⁺/CD11b⁺ DCs are involved in Th17 and Treg cell induction (19,51,56), CD103⁻/CD11b⁺ DCs play a role in Th17 cell induction (8,51), and CD103⁻/CD11b⁻ DCs are a factor in Th2 cell induction (55).

Adjuvant Sensing at the Mucosal Surface

Many cell surface and endosome innate immune receptors are characteristically expressed on limited cell types. For example, TLR3 is specifically expressed on CD8α⁺ DCs (12). In contrast, most cytosolic innate immune receptors, such as RLR, NLR, ALR, and cGAS/STING, are thought to be ubiquitously expressed by a variety of cell types. In addition to their basal expression, the expression levels of some innate immune receptors are upregulated by inflammatory situations (6). The mucosal epithelium expresses many innate immune receptors, including TLRs, and a variety of TLR agonists have been reported to work as mucosal adjuvants, including Muramyldipeptide, PolyIC, MPL, flagellin, and CpG (1,30,59,60). However, which TLRs are expressed in the mucosal epithelium is sometimes controversial and difficult to determine because epithelium sample preparation can easily become contaminated with other immune cells, such as macrophages and DCs, which can express various TLRs (1). In addition, many bacterial toxins, including cholera toxin and heat-labile enterotoxin, are well-known experimental mucosal adjuvants without being TLR agonists, but their mode of action is not yet well understood.

Furthermore, as mentioned above, nonmicrobial-derived products, including liposomes, oil emulsions, and several kinds of nanoparticles, also act as mucosal adjuvants. Some of these products function as a true delivery system, while others induce local and temporal mucosal epithelial damage or stress, which stimulates the release of DAMPs. The released DAMPs are then recognized by other epithelial cells and mucosal immune cells, including DCs, resulting in innate immune responses. It is likely that many mucosal adjuvants directly act on both epithelial and mucosal DCs, such that both contribute to the adjuvant effect. As discussed further below, flagellin works directly on the TLR-expressing epithelium, but TLR5 expression on the mucosal DC is not required for the adjuvanticity of flagellin in the nasal route (18,57), indicating that some of the effects of this mucosal adjuvant on mucosal DCs are indirect.

Mucosal Adjuvants and Their Modes of Action

A number of parental vaccine adjuvants also work as mucosal adjuvants, and many have been tested in animals via the intranasal route. In this study, some representative intranasal mucosal adjuvants are highlighted; they are categorized as (1) direct innate immune receptor agonist acting as PAMPs, (2) DAMP inducer, or (3) delivery systems, based on their apparent modes of action (Fig. 1).

PAMP adjuvants

Many microbe-derived substances, including TLR ligands, work as mucosal adjuvants through their specific innate immune receptor-mediated signaling in the host cells.

Bis-(3′,5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) is a bacterial intracellular signaling molecule that has been reported as a potent mucosal vaccine adjuvant for inducing Th1 and Th17 immune responses for a plant-derived H5 influenza vaccine (31). C-di-GMP is known as an agonistic ligand of STING, which recognizes cytosolic cyclic dinucleotides and activates the TBK1–IRF3 axis of IFN-I responses. Although the adjuvanticity of c-di-GMP as a mucosal adjuvant was completely lost in STING-deficient mice, the adjuvant effect of c-di-GMP is independent of IFN-I signaling and is dependent on the STING–NFκB axis of TNFα signaling in DCs (3). Blaauboer *et al.* further demonstrated that c-di-GMP, which is not cell membrane permeable, is taken up by pinocytosis-efficient DCs after nasal administration *in vivo* and stimulates antigen uptake in DCs and epithelial cells (4). They also found that c-di-GMP treatment *in vivo* can activate STING-independent signaling in cells other than DCs, resulting in IL-1, IL-6, IL-33, and TSLP secretion, however, the details of this c-di-GMP-mediated STING-independent IL-1α, IL-33, and TSLP secretion were not known (4). These data suggest that c-di-GMP works as a potent mucosal adjuvant by enhancing the whole process involved in DC-mediated antigen presentation to T cells, from the mucosal surface to the draining lymph nodes.

The TLR5 agonist flagellin is an effective mucosal vaccine adjuvant. TLR5 is specifically expressed on the subset of CD103⁺CD11b⁺ DCs, especially those in the intestinal lamina propria, and TLR5 signaling in this DC subset has been shown to play an important role in intestinal IgA production and Th17 differentiation (56). However, the mode of action of flagellin in the airway mucosa (which does not contain CD103⁺CD11b⁺ DCs) is not yet fully understood. Recent reports demonstrated that the mucosal adjuvant activity of flagellin does not require TLR5-expressing hematopoietic cells, including DCs. Instead, flagellin acts on the airway epithelial cells that express TLR5 (18,57). These studies suggest that flagellin first activates the airway mucosal epithelial cells and then indirectly activates the airway resident cDCs, which are essential to the flagellin mucosal adjuvant activity.

DAMP adjuvants

Innate immune receptors also recognize self-derived substances that are released or secreted on cellular stress or

death. Recent studies have suggested that some adjuvants act as DAMP adjuvants in the nasal mucosa.

Hydroxypropyl-β-cyclodextrin (bCD) is a commonly used excipient to solubilize pharmaceutical agents of poor solubility in water. Recent studies demonstrated that bCD works as a vaccine adjuvant when it is administered with vaccine antigen via a subcutaneous (43) or intranasal route (27). A local and temporal high concentration of bCD at the site of administration causes local cellular stress and death, resulting in host cell DNA release. This released DNA works as a DAMP to induce Th2 immune responses in a TBK1 signaling-dependent manner.

Endocine™ is a lipid-based mucosal adjuvant consisting of oleic acid and mono-olein, and it is under development as a nasal influenza vaccine adjuvant (32) for clinical use, however, the mechanisms of action were not known. A recent study demonstrated that although Endocine is a lipid adjuvant, neither TLR2 nor TLR4, either of which may recognize a lipid moiety in the adjuvant, is required for the adjuvanticity of Endocine in a mouse model. In an in vitro DC stimulation study, Endocine rapidly killed cultured DCs in a concentration-dependent manner. After intranasal administration, Endocine induced local cell death, which was associated with lactate dehydrogenase, DNA, and RNA release in the nasal wash (these are indicating cell death at the nasal mucosa, including many different cell types). Similar to bCD, the adjuvant activity of Endocine was dependent on a TBK1 signaling pathway, suggesting the involvement of DAMPs. Coadministration of RNase A, but not of DNase I, significantly reduced the Endocine-mediated antibody response; however, neither treatment reduced the cholera toxin-mediated antibody response, suggesting that the adjuvanticity of Endocine is characteristically mediated by host cell-released RNA as a DAMP (23).

Delivery system adjuvants

It also has been shown that enhancing nasal mucosal surface attachment by using cationic nanometer-sized gels or by applying bioengineered targeting of epithelial cell surface molecules is effective for augmenting mucosal immune responses, and, in many cases, these approaches seem not to require additional PAMP/DAMP adjuvants.

Cholesteryl group-bearing pullulan (CHP) is a self-assembled polymer-based nanoparticle carrier used to deliver drugs or vaccine antigens. Nochi et al. demonstrated that with botulinum antigen encapsulation, which they called CHP-BoHc/A, the CHP-BoHc/A did not show any improvement compared with BoHc/A alone. However, cationic CHP with BoHc/A (cCHP-BoHc/A) showed significant induction of antigen-specific local and systemic IgA and IgG responses. This improvement was associated with prolonged ($\sim$10 h) retention of cCHP-BoHc/A in the nasal cavity. Epithelial cell-attached cCHP-BoHc/A was internalized by both epithelial cells and M cells, and the antigen was subsequently taken up by CD11c$^+$ DCs. Interestingly, increased DC maturation was not observed in either in vitro or in vivo experiments. The authors concluded that effective antigen delivery through the mucosa via cCHP is sufficient for the induction of mucosal immune responses (41).

FcRn is a receptor of IgG that allows fetuses to obtain maternal IgG through the placental and intestinal routes, and it has been observed that FcRn can transport IgG across mucosal epithelia in adults. Ye et al. reported that intranasal immunization using HSV-2 glycoprotein gD fused with the IgG Fc fragment acts as a protective mucosal vaccine against intravaginal virulent HSV-2 186 strain challenge. They demonstrated that this was mediated by FcRn using gD fused with mutant Fc, which lacked FcRn binding, or with FnRn knockout mice. The authors concluded that Fc-fused antigens are efficiently transported by nasal epithelial cells in an FcRn-dependent manner and are subsequently transferred to mucosal antigen-presenting cells, such as DCs (63).

The C-terminal fragment of Clostridium perfringens enterotoxin (C-CPE) has a high binding affinity for nasal epithelial cells expressing claudin-4. Bioengineering of a vaccine antigen fused with C-CPE works as another vaccine antigen targeting method for the nasal mucosal epithelium. Suzuki et al. demonstrated that pneumococcal surface protein A (PspA) fused with C-CPE induced protective systemic and respiratory antibody responses in mice, which are associated with PspA-C-CPE binding to the nasal epithelium and M cells (53).

Context-Dependent Mucosal Immune Responses

Although each DC subset has specialized default functions, several recent reports have suggested that its functions are also regulated by the associated immune context. DePaolo et al. demonstrated that retinoic acid, which is reported to play a critical role in Treg differentiation through CD103$^+$ DCs in the intestine, also acts as an adjuvant for inducing CD4$^+$ T cell-mediated autoimmunity against fed antigens when IL-15 is present (13). These findings suggest that the presence of IL-15 regulates tolerance or immunity. In addition, Yang et al. showed that colonization with segmented filamentous bacteria (SFB) induced a Th17 response to the antigen derived from SFB; in contrast, colonization with Listeria monocytogenes expressing this SFB-derived antigen, elicited Th1 instead of Th17 responses (62). It has been known that L. monocytogenes infection typically induces Th1 responses, suggesting that the bacterial host, rather than antigen itself, affects Th differentiation. More recently, Stary et al. used a vaginal mucosal vaccine model for Chlamydia trachomatis (Ct) infection in mice and demonstrated that ultraviolet (UV) light-inactivated Ct (UV-Ct) generated Tregs via CD103$^+$ DC-mediated antigen presentation resulting in nonprotective vaccination, whereas vaccination with UV-Ct plus R848 containing a PLGA particle adjuvant, which the authors called charge-switching synthetic adjuvant particles (cSAP), activated Ct-specific protective CD4$^+$ T cell responses via CD103$^-$ DCs. This protective immunity was similar to that induced by vaccination with live Ct at the vaginal mucosa (52), suggesting that the UV-Ct-associated innate immune signature promotes Tregs, while the cSAP adjuvant promotes effector CD4$^+$ T cells. These reports strongly suggest that antigen presentation by mucosal DC subsets is profoundly influenced by aspects of the surrounding immune environment, such as the presence of particular cytokines, the antigen providing platform, and the specific adjuvant.

Importance of Selecting an Appropriate Adjuvant and Drug Delivery System

The development of vaccine antigen purification/production techniques and drug delivery systems has enabled a variety of combinations of antigen and adjuvant, including totally artificial antigen and adjuvant combinations. Maroof *et al.* reported an insightful example for a split influenza vaccine with a synthetic TLR4 agonist, CRX-601, as an exogenous synthetic adjuvant. Usually, live influenza virus infection and whole inactivated influenza vaccine induce Th1 responses, whereas immunization with this split vaccine induced Th2 responses, and these influenza virus-specific Th1 and Th2 responses are both protective against influenza virus infection (22,26). Interestingly, intranasal, but not subcutaneous, split vaccine immunization with the CRX-601 adjuvant promoted influenza-specific Th17 responses, and these immunization route- and adjuvant-dependent Th17 responses are detrimental for influenza virus challenge through an IL-17A-mediated augmentation of neutrophilic lung inflammation (33). This indicates that at least when considering a mucosal vaccination, the use of Th17-inducing adjuvants should be avoided for influenza split antigen vaccines. Furthermore, the selection of both an appropriate adjuvant and administration route (or drug delivery system) is important for making an effective mucosal vaccine.

Conclusion

A variety of substances can act as mucosal adjuvants; however, their modes of action are very different. Some directly act on specialized DC subsets, while others work indirectly. Nonmicrobial-derived substances can also function as mucosal adjuvants. Some of these substances act purely as a delivery system; others are DAMP inducers, which indirectly induce innate immune responses. This DAMP inducer-mediated epithelial cell damage may also break up the mucosal barrier that otherwise prevents vaccine antigen and adjuvant from reaching the mucosal lamina propria where important DC subsets reside. Which types of adjuvant are appropriate is dependent on the target disease, population, and the selected antigen. In some cases, the addition of an adjuvant that induces the same Th type as the original disease antigen is optimal, but in other instances, it may be better to use an adjuvant that induces a different Th type. The selection of adjuvants must aim to appropriately balance safety and efficacy; however, there are not yet any clear cut rules to guide these choices. Further progress at the molecular level of vaccine science will make it possible to produce much safer and more effective mucosal vaccines and vaccine adjuvants in the future.

Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Dr. Taiki Aoshi
Vaccine Dynamics Project
BIKEN Innovative Vaccine Research Alliance Laboratories
Research Institute for Microbial Disease
Osaka University
3-1 Yamadaoka, Suita
Osaka 565-0871
Japan

E-mail: aoshi@biken.osaka-u.ac.jp