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The development of mucosal vaccines for both mucosal and systemic immune induction and the roles played by adjuvants

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Vaccination is the most successful immunological practice that improves the quality of human life and health. Vaccine materials include antigens of pathogens and adjuvants potentiating the effectiveness of vaccination. Vaccines are categorized using various criteria, including the vaccination material used and the method of administration. Traditionally, vaccines have been injected via needles. However, given that most pathogens first infect mucosal surfaces, there is increasing interest in the establishment of protective mucosal immunity, achieved by vaccination via mucosal routes. This review summarizes recent developments in mucosal vaccines and their associated adjuvants.

Keywords: Adjuvants, Mucosal immunity, Vaccines

Introduction

Early vaccination strategies used live or attenuated pathogens to induce adaptive immunity [1]. However, the re-activation of attenuated vaccine materials and the possible tumorigenicity of inactivated oncogenic viruses are important safety concerns [2]. Thus, non-pathogenic materials including inactivated toxins, synthetic peptides, and recombinant subunit proteins have been considered as vaccine materials [3]. However, these antigens are poor in immunogenicity and must be given with supplemental materials to potentiate the vaccination capacity [3]. Such supplemental materials are termed adjuvants and can be divided into two classes depending on their mode of action: efficient delivery of vaccine materials and/or stimulation of the immune system (Tables 1, 2) [1]. Adjuvants facilitating vaccine delivery include liposomes, nanogels, oil-in-water emulsions, and virosomes targeting the co-administered antigens to professional antigen-presenting cells (APCs) [4]. Adjuvants that stimulate the immune system include molecules binding to intracellular receptors including Toll-like receptors (TLRs), Nod-like receptors, and RIG-I–like receptors and to cytosolic DNA sensors, all of which modulate the immune response [1]. However, most adjuvants have been evaluated in the context of parenteral immunization; thus, it is not clear how well the adjuvants function in the mucosal immune compartment.

The Mucosal Immune System

Mucosal surfaces cover 400 m^2 of the body including the gastrointestinal, urogenital,

Table 1. Currently licensed adjuvants used as carriers of vaccine materials

Adjuvant name	Adjuvant class	Immune response	Component
Alum	Mineral salts	Antibody, Th2 response	Aluminum phosphate or aluminum hydroxide
MF59	Oil-in-water emulsion	Antibody, Th1/Th2 response	Squalene, Polysorbate 80 (Tween 80), sorbitan trioleate (Span 85)
Virosomes	Liposomes	Antibody, Th1/Th2 response, cross-presentation	Lipids, hemagglutinin
AS03	Oil-in-water emulsion	Antibody, Th1/Th2 response	Squalene, Polysorbate 80 (Tween 80), α-tocopherol
Montanide ISA51	Water-in-oil emulsion	Antibody, Th1/Th2 response	Drakeol 6 VR, mannide monooleate

Based on Rappuoli R et al. Nat Rev Immunol 2011;11:865-72 [1].

Table 2. Immunostimulatory molecules used as vaccine adjuvants

Adjuvant name	Target receptor	Type (component)	Immune response
Licensed adjuvant			
RC529	TLR4	RC529	Antibody, Th1 response
AS01	TLR4	Liposome, MPL, QS21	Antibody, Th1 response, $CD8^+$ T cells
AS04	TLR4	Aluminum hydroxide, MPL	Antibody, Th1 response
Not licensed adjuvant			
Poly(I:C), Poly(IC:LC)	TLR3	dsRNA	Type I IFN, pro-inflammatory cytokines, antibody, CD4/CD8 response
Imiquimod, Resiquimod, Gardiquimod	TLR7/TLR8	ssRNA	Type I IFN, pro-inflammatory cytokines, antibody, CD4/CD8 response
IC31	TLR9	Unmethylated CpG DNA	Type I IFN, pro-inflammatory cytokines, antibody, CD8 response
iE-DAP, MDP	NOD1/2	Peptidoglycan	Pro-inflammatory cytokines, antibody
M8, defective interfering (DI) RNA	RIG-1, MDA-5	dsRNA	Type I IFN, pro-inflammatory cytokines, antibody, CD4/CD8 response
cGAMP, C-di-GMP	STING	Cyclic dinucleotide	Type I IFN, pro-inflammatory cytokines, antibody, CD8 response

Based on Rappuoli R et al. Nat Rev Immunol 2011;11:865-72 [1].
TLR, Toll-like receptor; IFN, interferon.

and respiratory tracts [5]. Mucosae are continuously exposed to microbiota and antigens. The gastrointestinal mucosa is especially prone to the development of tolerogenic microenvironments, where luminal antigens may persist. The mucosal immune system has both inductive and effector sites differing in terms of their anatomical and functional characteristics [6]. The major mucosal immune inductive sites include gut-associated lymphoid tissue (GALT) and the nasopharyngeal-associated lymphoid tissue (NALT). GALT includes Peyer's patches, mesenteric lymph nodes, and isolated lymphoid follicles, while NALT includes tonsils/adenoids, inducible bronchus-associated lymphoid tissue, cervical lymph nodes, and hilar lymph nodes. Mucosal immune inductive sites are covered by follicle-associated epithelium (FAE), which is composed of enterocytes and M cells.

M cells are specialized epithelial cells for antigen uptake [7]. These cells are overlaid by a thin mucus layer and possess short irregular microvilli [8]. M cells can transfer antigens via transcytosis to APCs located in pockets within M cell clusters [9]. Dendritic cells that come in contact with antigens transcytosed through M cells enter the interfollicular T cell zone to activate naïve T cells [10]. Finally, effector T cells move to the B cell follicles of germinal centers (GCs) and secrete cytokines capable of promoting IgA class-switch recombination [11]. In mucosal immune effector sites such as the lamina propria of the gut, the upper respiratory tract, and the female reproductive tract, IgA^+ plasma cells terminally differentiate to release secretory IgA (SIgA), the most important immune effector molecule in the mucosa. SIgA is transported across mucosal epithelial cells via a polymeric Ig receptor (pIgR) (Fig. 1) [12]. SIgA is a major immune effector at mucosal surfaces that acts via three mechanisms: antigen excretion, immune exclusion, and intracellular antigen neutralization (Fig. 2) [13]. Antigen excretion by SIgA features the binding of SIgA to pathogen-derived antigens, thus inhibiting pathogen–epithelial cell contact. SIgA exerts immune exclusion by eliminating antigens via secretion of an IgA–antigen complex, and invading pathogens can also be eliminated by complex formation with IgA-joining (J) chain-pIgR. SIgA inhibits the binding of pathogens and/or pathogenic antigens to specific receptors by neutralizing and eventually removing the pathogenic antigens.

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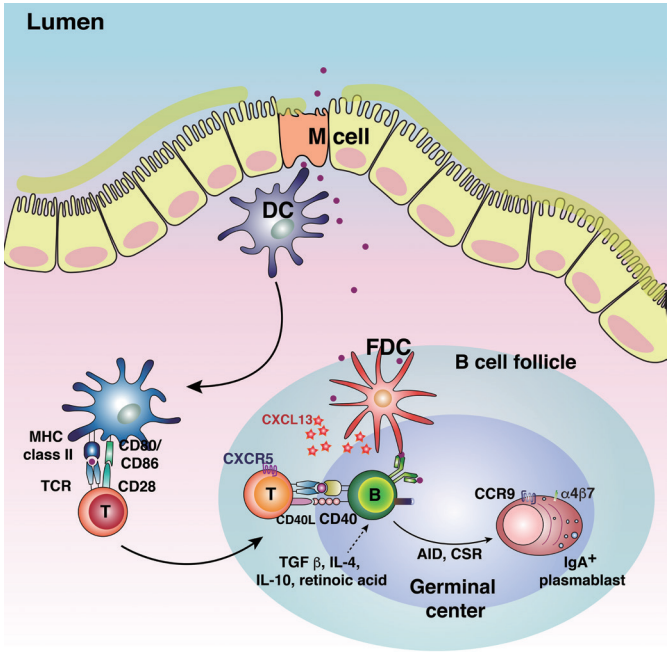

Fig. 1. Schematic diagram of mucosal immune induction. The luminal antigens transcytosed by M cells encounter dendritic cells (DCs) in the subepithelial dome of Peyer's patch. DCs loaded with the antigens move into the interfollicular T cell zone and induce the effector T cells. Antigen-specific effector $CD4^+$ T cells expressing CD40 ligand induce the IgA^+ plasmablasts. FDC, follicular dendritic cell; TCR, T-cell receptor; TGF β, transforming growth factor β; IL, interleukin; AID, activation-induced cytidine deaminase; CSR, class switch recombination; CXCL13, CXC chemokine ligand 13; CXCR5, CXC chemokine receptor 5.

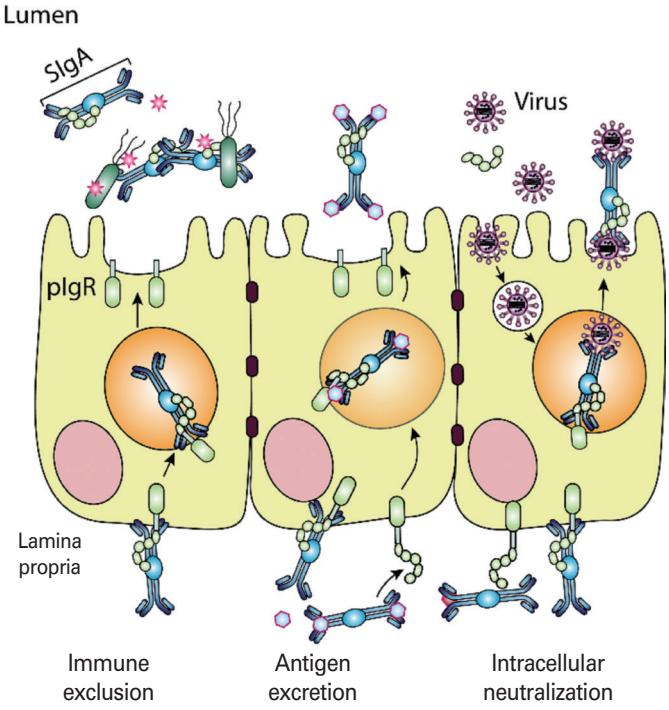

Fig. 2. The role played by secretory antibodies in the mucosal compartment. Secreted antibodies can protect mucosal surfaces by immune exclusion, antigen excretion, and intracellular neutralization. Immune exclusion is that secretory IgA (SIgA) interact with antigens and block their attachment to epithelial cells. The SIgAs bind to antigen and remove from the lamina propria through antigen excretion. The intracellular pathogen can also be eliminated by intracellular neutralization.

Mucosal Vaccines and Delivery Routes

Mucosal vaccination can induce antigen-specific humoral and cell-mediated immune responses in both the systemic and mucosal compartments [14]. Additionally, such vaccination efficiently induces long-lasting B- and T-cell memory [15]. Importantly, the characteristics of mucosal immune response induction depend on the vaccine delivery route chosen (Fig. 3). For example, oral delivery (a traditional form of mucosal vaccination) can induce production of antigen-specific SIgA in the gastrointestinal tract, salivary glands, and mammary glands [14]. Currently, the licensed human live attenuated vaccines for rotavirus, poliovirus, *Salmonella* Typhi, and cholera are delivered orally (Table 3) [14,16]. Intranasal vaccines such as FluMist, a live attenuated influenza virus vaccine, generate SIgA in the upper and lower respiratory, gastric, and genital tracts [17]. Upon sublingual vaccination, antigen-specific immune responses are induced in the gastrointestinal and the upper and lower respiratory tracts [14].

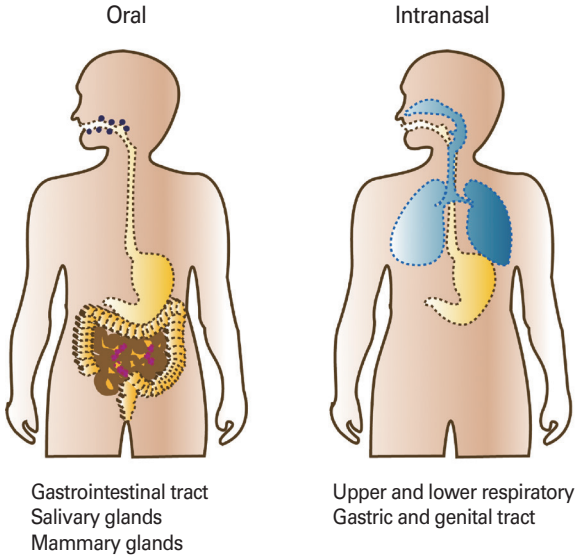

Fig. 3. Mucosal immunization routes and the regions affected. The mucosal IgA responses are differentially induced according to the routes of mucosal immunization. Oral vaccination is effective for the immune induction in the gastrointestinal tract, salivary glands, and mammary glands. Intranasal vaccination is effective for the immune induction in respiratory, gastric and genital tracts.

Table 3. Currently licensed mucosal vaccines

Pathogen	Trade name	Composition	Dosage	Immunological mechanism	Efficacy
Rotavirus	Rotarix, RotaTeg	Live attenuated, monovalent or pentavalent rotaviruses	Oral, 3 doses	Mucosal IgA and systemic neutralizing IgG	Over 70%-90% against severe disease
Poliovirus	Orimune, OPV, Poliomyelitis vaccine	Live attenuated trivalent, bivalent, and monovalent polioviruses	Oral, 3 doses	Mucosal IgA and systemic IgG	Over 90% in most of the world
Salmonella Typhi	Vivotif, Ty21A	Live attenuated *S.* Typhi *bacteria*	Oral, 3-4 doses	Mucosal IgA, systemic IgG, and CTL responses	Variable, but more than 50%
Vibrio cholera	Dukoral, ORC-Vax, Shanchol	Inactivated *V. cholera* O1 classical and El Tor biotypes with or without CTB	Oral, 2-3 doses	Antibacterial, toxin-specific, and LPS-specific IgA	Strong herd protection over 85%
Influenza type A and B virus	FluMist	Live viral reassortant with trivalent mix of H1, H3, and B strains of hemagglutinin and neuraminidase genes in an attenuated donor strain	Intranasal in young children, 2 doses	Hemagglutinin- and neuraminidase-specific mucosal IgA and systemic IgG responses	>85% in children, variable in adults

Adapted from Lycke N. Nat Rev Immunol 2012;12:592-605 [14] and Kim SH and Jang YS. Exp Mol Med 2014;46:e85 [16].
OPV, oral polio vaccine; CTL, cytotoxic T lymphocytes; CTB, cholera toxin B subunit; LPS, lipopolysaccharide.

Table 4. M cell–specific molecules and their ligands

Ligand	Receptors on M cells	Reference
Ulex europaeus 1 (UEA-1)	α1,2 fucose	[21]
Aleuria auranitia (AAL)	α-L-fucose	[20]
Galectin-9	N-glycans/repeated oligosaccharide	[22]
Peptide Co1 (SFHQLPARSPLP)	C5aR	[23]
Cathelicidin LL-37	P2X7 receptor, Formyl peptide receptor 2	[25] [24]
Antibody NKM 16-2-4	α1,2 fucose-containing carbohydrate	[33]
Antibody LM112	Sialyl Lewis A	[29]
Antibody 3G7-H9	Glycoprotein 2	[27]
σ1 protein (reovirus)	α2,3 sialic acid	[39]
Invasion (*Yersinia*)	β1 integrin	[26]
Long polar fimbriae (*Escherichia coli, Salmonella*)	Unknown	[29]
FimH (*E. coli, Salmonella*)	Glycoprotein 2/Uromodulin	[36]
OmpH (*Yersinia*)	C5aR	[23]
LPS	TLR-4	[28]
Lipoteichoic acid	TLR-2	[37]
Phosphorylcholine moiety of LPS	PFAR	[38]
Hsp60 of *Brucella abortus*	Cellular prion protein	[32]
Lipid A domain of LPS (gram-negative bacteria)	ANXA5	[35]
Bacterial peptidoglycan	PGLRP-1	[34]
SIgA	Unknown	[30]
c-term domain of enterotoxin (*Clostridium perfringens*)	Claudin 4	[31]

Adapted from Kunisawa J, et al. Adv Drug Deliv Rev 2012;64:523-30 [29] and Kim SH and Jang YS. Exp Mol Med 2014;46:e85 [16].
LPS, lipopolysaccharide; Hsp60, heat shock protein 60; SIgA, secretory IgA.

Although a few human mucosal vaccines are licensed, safety issues remain; the current vaccines are live attenuated or non-living whole-cell vaccines (Table 3). Subunit vaccines lacking entire pathogens are considered to be safer next-generation vaccines. However, several issues must be addressed when developing subunit mucosal vaccines, including poor immunogenicity, degradation of vaccine materials in the harsh mucosal environment, delivery of vaccine materials to mucosal immune inductive tissue, and modulation of the mucosal immune environment such that oral tolerance does not develop.

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Table 5. Mucosal adjuvants

Composition	Target	T-cell–mediated immune response				Mucosal IgA	Reference
		Th1	Th2	Th17	CTL		
MDP	TLR-2	+	+	-	-	+	[37]
MPL	TLR-4	+	-	-	+	+	[45]
Flagellin	TLR-5	+	-	-	+	++	[47]
CT	GM1	-	+	+	+	++++++	[42]
CTA1-DD	Ig heavy chain	+	+	+	+	+++++	[41]
Quillaja saponins fraction	DCs	+	+	-	+	++	[44]
Cationic DDA	ND	+	-	-	+	++	[43]
Chitosan	Tight junctions	-	+	-	-	++	[48]
IL-1	IL-1R	+	+	-	-	+++	[46]
IL-12	IL-12R	+	-	-	+	+	[49]

Adapted from Lycke N. Nat Rev Immunol 2012;12:592-605 [14] and Kim SH and Jang YS. Exp Mol Med 2014;46:e85 [16].
+ or - in this table means the strength of induced immune response.
CTL, cytotoxic T lymphocytes; MDP, muramyl dipeptide; TLR, Toll-like receptor; MPL, monophosphoryl lipid A; CT, cholera toxin; DC, dendritic cell; DDA, dimethyldioctadecylammonium; ND, not determined; IL, interleukin.

Mucosal Vaccine Adjuvants

The mucosa is continuously exposed to various antigens and microbiota and tightly regulates the influx of luminal antigens. Therefore, special delivery systems are required for development of successful mucosal vaccines [18]. M cells are the ideal targets of mucosal vaccine materials. Not only are the cells localized to the FAE of mucosal immune inductive sites, but many APCs are located nearby and/or under pockets of M cells. Although antigen uptake by M cells was previously thought to be non-specific, many recent studies have shown that a specific antigen delivery mechanism is involved [19]. GP2, a protein expressed specifically by M cells, drives transcytosis of $FimH^+$ bacteria into such cells. Therefore, M cell-specific markers can be utilized for antigen delivery to mucosal immune inductive sites [20-39] (Table 4). For example, an M cell-specific antibody, NKM 16-2-4, recognizes the α(1,2)-fucose-containing carbohydrate moiety of M cells and can be used to enhance delivery of an associated antigen [33]. Additionally, an M cell-targeting ligand, Co1, also targets antigens to M cells by interacting with the complement 5a receptor, inducing an antigen-specific immune response [23,40]. Thus, M cell–targeting of vaccine materials will play a pivotal role in successful mucosal vaccination.

In the tolerogenic mucosal environment, adjuvants with immunostimulatory capacities enhance immune induction (Table 5) [37,41-49]. When TLR agonists such as Pam3CSK4, poly(I:C), MPL, or CpG-ODN were given either nasally or orally, in combination with vaccine materials, both systemic and mucosal antigen-specific immune responses were enhanced [3]. In addition, some immunostimulatory adjuvants improve the quality of the immune response. Cholera toxin (CT) is an effective mucosal vaccine adjuvant because it interacts with the GM1 ganglioside. However, the use of CT in this context raises a safety concern. Thus, CTA1-DD, which contains a mutant GM1 ganglioside-targeting A subunit of CT and the D-fragment of *Staphylococcus aureus* protein A to activate follicular dendritic cells (FDCs) closely associated with GCs, has been developed. CTA1-DD effectively promotes the induction of high-affinity B-cell clones and long-lived memory B cells and plasma cells [50]. Another mucosal vaccine adjuvant is the oil-in water emulsion MF59, which is currently licensed for human use. Although the mechanism of action remains unclear, MF59 not only enhances recruitment of innate immune cells via release of ATP and antigen uptake, but it also increases the adjunctive capacities of B cells by enhancing GC actions via activation of follicular helper T cells [51]. Finally, cathelicidin LL-37 is an immunostimulatory adjuvant that targets antigens to M cells. LL-37 increases antigen delivery to such cells and activates FDCs by interacting with the formyl peptide receptor 2 [24]. This enhances the induction of antigen-specific immune responses in both the systemic and mucosal compartments.

Conclusion

Recently, the need for mucosal vaccines has become recognized. Such vaccines offer several advantages including safe-

ty, convenience of vaccination, economical production, induction of mucosal immune responses, and enhanced memory B- and T-cell induction. However, several hurdles must be overcome in the development of practical subunit mucosal vaccines, including poor immunogenicity, degradation of vaccine materials in a harsh mucosal environment, delivery of vaccine materials to mucosal immune inductive tissue, and modulation of the mucosal immune environment to ensure that oral tolerance does not develop. These obstacles will be overcome by developing effective mucosal adjuvants that target M cells and are immunostimulatory.

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