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Inducing mucosal immunity by systemic immunization

Induction of mucosal immunity through systemic immunization: phantom or reality?

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Abstract

Generation of protective immunity at mucosal surfaces can greatly assist the host defense against pathogens which either cause disease at the mucosal epithelial barriers or enter the host through these surfaces. Although mucosal routes of immunization, such as intranasal and oral, are being intensely explored and appear promising for eliciting protective mucosal immunity in mammals, their application in clinical practice has been limited due to technical and safety

related challenges. Most of the currently approved human vaccines are administered via systemic (such as intramuscular and subcutaneous) routes. Whereas these routes are acknowledged as being capable to elicit antigen-specific systemic humoral and cell-mediated immune responses, they are generally perceived as incapable of generating IgA responses or protective mucosal immunity. Nevertheless, currently licensed systemic vaccines do provide effective protection against mucosal pathogens such as influenza viruses and *Streptococcus pneumoniae*. However, whether systemic immunization induces protective mucosal immunity remains a controversial topic. Here we reviewed the current literature and discussed the potential of systemic routes of immunization for the induction of mucosal immunity.

Keywords

Mucosal immunity, IgA, systemic immunization, adjuvant

Introduction

The vast mucosal surfaces covering the gastrointestinal, urogenital and respiratory tracts, as well as the conjunctiva, inner ear and ducts of the exocrine glands, are endowed with powerful mechanical and physicochemical mechanisms that either prevent the entry of foreign bodies (including microorganisms) or facilitate their degradation.[1, 2] Highly specialized innate and adaptive mucosal immune responses at these surfaces are of major importance to modulate the colonization of commensal and pathogenic microorganisms, and to defend the host against the extravasation of the pathogens through the epithelium to cause diseases at other tissues.[3] Extensive research has demonstrated that secretory IgA is the main immunoglobulin isotype mediating humoral immunity at mucosal surfaces, but some studies have shown that locally produced IgM and IgG also contribute to the mucosal immune defense.[4-10] Therefore, vaccines that generate protective antibody (and cell-mediated) responses at mucosal sites would greatly advance the field of vaccinology.

Mucosal route of immunization elicits immune responses at the local and distal mucosal sites, as well as systemic immune responses. Therefore, most current efforts attempting to elicit protective mucosal immunity have focused on the mucosal (such as oral and intranasal) routes of vaccination. Although live, attenuated oral vaccines are generally immunogenic and induce excellent

protective immunity against the targeted pathogen, the production of such vaccines are complex and need to grow large amounts of the pathogen prior to their attenuation. The use of nonpathogenic mutants is relatively safer, but suffers from the potential risk of reversion to virulence. In contrast, non-replicating mucosal vaccines, based on subunit or acellular antigens, would be preferable from safety perspectives. However, subunit oral vaccines require administration of relatively large amounts of antigens to compensate for antigen degradation in the gastrointestinal tract, the co-administration of potent adjuvants and/or delivery system to facilitate antigen uptake by the antigen presenting cells (APC), and the need for neutralization of stomach acids prior to vaccine administration.[11] In contrast, the intranasal (i.n.) route of immunization requires lesser amounts of antigens than the oral administration, but the safety and efficacy of i.n. vaccines remain to be established.[12-14] For example, the currently licensed influenza vaccine, FluMist, is not recommended for children aged <2 yr or children aged <5 yr with a history of recurrent wheezing, or for asthmatic children and adults of any age.[15] Although great advances have been made towards the development of safe and effective subunit mucosal vaccines, there has been a renewed interest in investigating the potential of systemic immunization for eliciting mucosal immunity.

Systemic immunization has generally been considered as incapable of generating

protective mucosal immune responses for a longtime now, however, cumulative data from recent studies suggest that some systemically administered vaccines are capable of eliciting mucosal immune responses, including secreting IgA antibodies. Such systemic vaccines may offer potential manufacturing and regulatory advantages over the mucosal vaccines. Here, we reviewed the current literature and discussed the potential of systemic routes of immunization with non-replicating vaccines for inducing mucosal immunity in mammalian hosts. We have also included some references to transcutaneous immunization (TCI) in this review, since many of these studies involve the use of micro-needles or other means to penetrate past the intact skin surface to deliver the vaccine to the epidermis.

Intraperitoneal immunization

Intraperitoneal (i.p.) administration of vaccines has long been used and studied as an experimental immunization route for the induction of systemic immunity in animal models of vaccination. However, the i.p. route, in certain antigen-adjuvant combinations, has also been reported to induce mucosal immune responses, particularly gastrointestinal IgA responses. For example, i.p. immunization of an inactivated poliovirus vaccine with 1,25-Dihydroxyvitamin D_3 as an adjuvant significantly promoted not only serum IgG but also salivary IgA responses in mice.[16] Robust antigen-specific serum IgG and pulmonary IgA

responses were generated in pigs upon i.p. immunization with a *Mycoplasma hyopneumoniae* antigen co-administered with an oil emulsion.[17] The i.p. administration of *Bacillus thuringiensis* Cry1Ac protoxin in mice generated high levels of IgG and IgM, and low but detectable levels of IgA in sera and the lavage fluids from various mucosal sites (vagina, respiratory tract, small and large intestine).[18] The magnitude of individual Ig isotype responses induced appears to be depended on the mucosal site analyzed, with IgA being the highest in small intestine and both IgG and IgM being the strongest in respiratory tract. Although the protective efficacies of the induced mucosal immune responses were not evaluated in this study, a subsequent study by this group has shown that the mucosal immune responses elicited by i.p. immunization with the Cry1Ac protoxin and amoebal lysates enhances the protection against lethal intranasal challenges with *Naegleria fowleri* in mice.[19] Similarly, i.p. administration of an inactivated SARS Coronavirus (SARS-CoV) vaccine adjuvanted with a Poly (I:C) derivative induced antigen-specific IgG and IgA responses at multiple mucosal sites in mice, with the highest levels in the intestine and less significant but robust responses in vaginal washes and lowest responses in the mouth/saliva, while only strong IgG but no IgA responses were observed in sera and lungs.[20] Moreover, those systemic and mucosal antibodies were effective in virus neutralization activity.[20] In contrast, i.p. immunization of mice with

mycobacterium PstS-1 antigen failed to induce any specific IgA responses in bronchoalveolar lavage (BAL) or saliva, nor did it induce cytokine responses (e.g., IL-4, IL-5 and IFN-γ) in the lungs, although strong serum IgG responses were observed.[21] In another study, little protection was observed against pulmonary infection in mice after i.p. vaccination with a cholera toxin (CT)-adjuvanted *Mycoplasma pulmonis* vaccine.[22] In a clinical study, i.p. immunization of patients on continuous ambulatory peritoneal dialysis with tetanus toxoid elicited significant specific IgG and IgA responses in sera and peritoneal fluids, and salivary IgG but failed to induce secretory IgA responses.[23] The inefficiency of i.p. immunization in generating mucosal immune responses was also observed in several other studies.[24, 25]

However, virus-like particles (VLPs) delivered by i.p. route have shown good potential in generating both systemic and mucosal immune responses. For example, i.p. administration of mice with CpG-adjuvanted SARS-CoV VLPs increased antigen-specific IFN-γ and IL-4 producing cell populations in the spleen, and IgA antibodies in lungs, intestine, feces, and vaginal washes.[26] Interestingly, i.p. immunization of mice with rotavirus 2/6 VLPs was shown to be more effective than oral immunization in the induction of mucosal IgG and IgA in the feces and uterine fluids, and serum IgG responses.[27] Furthermore, it is interesting to note that i.p. immunization with HIV-1 VLPs could induce

significant cross-clad neutralizing antibodies against both autologous and heterologous primary isolates in sera and vaginal washes, and elicit stronger cytotoxic T lymphocyte (CTL) responsesthani.n.immunization.[28]

Intramuscular immunization

Intramuscular (i.m.) administration is the most predominant vaccine delivery method for humans, and it enables relatively larger volumes to be injected.[29] In addition, i.m. immunization has been widely used in the immunogenicity and efficacy studies of experimental DNA vaccines. Those studies have demonstrated that i.m. vaccination can promote both systemic and mucosal immune responses, and protect against mucosal pathogen challenge.[30-34] For example, i.m. immunization with anti-caries DNA vaccine encoding *S. mutans* antigens fused to cytotoxic T lymphocyte antigen-4 (CTLA-4) elicited strong serum IgG and salivary IgA responses in both rabbits and monkeys.[35] Moreover, i.m. immunization of two-week-old calves with a bovine respiratory syncytial virus (BRSV) DNA vaccine induced antigen-specific IgG and IgA responses in sera and BAL fluids, and accorded protection against i.n.BRSV challenges.[36] More importantly, i.m. immunization of a bovine rotavirus VP6 DNA vaccine effectively protected mice against oral challenges with a murine rotavirus strain by reducing virus shedding in feces, suggesting that heterologous protection can be obtained by i.m. immunization of VP6 DNA vaccine.[37] Heterologous

protection was also observed against i.n. H5N1 challenge in ferrets i.m. immunized with H1N1 VLPs.[38] However, in mice only homologous protection was observed. In a human trial involving 6 healthy female volunteers, i.m. immunization with an alum-adjuvanted human papilloma virus (HPV) vaccine increased the numbers of circulating IgG- and IgA-secreting cells (ASCs) and generated HPV-specific IgG and neutralizing antibodies in sera, and cervical and vaginal wash fluids,[39] in consistence with the previous work where women i.m, immunized with HPV16 VLPs in menstrual cycle developed antigen-specific IgG in cervical secretions. [40] Furthermore, it was found that i.m. vaccination with an inactivated influenza virus elicited wide dispersion of IgG memory B cells to secondary lymphoid tissues including Peyer's patches (PP) and the nasal-associated lymphoid tissues, which would ensure prompt activation in the event of influenza infection.[41] In addition, i.m. vaccination of humans with the licensed inactivated hepatitis A and B vaccines induced high levels of specific antibody responses in sera and protection against hepatitis A and B infection,[42-45] Moreover, a recent meta-analysis of clinical studies indicate that i.m. immunization of >10-wk-old infants with two full or 1/5 doses of inactivated poliovirus vaccine resulted in >80% seroconversion and is likely to protect >80% of vaccinees against poliomyelitis.[46]

In addition to promoting robust antibody responses, i.m. immunization has been

shown to induce cell-mediated immune (CMI) responses at mucosal sites. For instance, i.m. immunization of mice with a DNA vaccine co-delivered with CCL25 chemokine enhanced antigen-specific IFN-γ secretion by $CD3^+CD8^+$ and $CD3^+CD4^+$ T cells in mesenteric lymph nodes (MLNs), and conferred complete protection against a lethal i.n. influenza challenge.[47] Similarly, i.m. administration of retinoic acid to mice immunized with a replication-defective adenovirus vector increased both effector and memory T cell numbers in the intestinal mucosal tissue and protected mice from an intravaginal vaccinia virus challenge.[48] Moreover, i.m. immunization of 7-day-old pigs with an inactivated *M. hyopneumoniae* vaccine significantly increased the number of IL-12 and IL-10 secreting cells in the lungs and bronchial lymph nodes, and generated antigen-specific IgG, IgM and IgA antibodies in BAL fluids as well.[49] In Indian rhesus macaques, a plasmid DNA vaccine expressing several SIV antigens delivered by i.m. electroporation increased antigen-specific IFN-γ-secreting, but not IL-2-secreting,T cells in blood and BAL fluids, with a greater proportion of specific $CD8^+$ T cells in BAL fluids than that in the blood.[50] Furthermore, a fourth i.m. immunization administered 90 weeks after the third one, rapidly boosted antigen-specific humoral and cellular responses with higher population of specific IFN-γ+ memory T cells in the BAL fluid than in the blood. On the other hand, some vaccines administered by i.m. route were less effective or

inefficient in inducing mucosal immune responses.[51, 52] For example, in the herpes simplex virus type 2 (HSV-2) vaccine trials, i.m. vaccination of subunit vaccines such as glycoprotein B in oil-in-water adjuvant and glycoprotein D in alum and 3-O-deacylated monophosphoryl lipid A, failed to protect against genital HSV-2 infection despite the good immunogenicity.[53, 54] Moreover, i.m. vaccination of the nursing home residents (aged 60–82 years) with an inactivated commercial influenza vaccine failed to elicit IgA responses in nasal washes, although strong haemagglutination inhibition (HI) titers were detected in the sera.[55] It is possible that the age or sex of the vaccinees or the type of vaccine administered was a contributing factor to these observations.

Subcutaneous immunization

Subcutaneous (s.c.) route of immunization is another conventional vaccination route widely used for various human vaccines and experimental vaccines in animal models. Recent studies suggest that s.c. immunization of non-replicating vaccines could induce both systemic and mucosal antigen-specific antibody responses, and protect the vaccinated animals against infectious challenge.[56-58] In a macaque study, s.c. immunization with HIV gp140 with recombinant macaque major histocompatibility complex (MHC) class I and II elicited serum and mucosal (recta and vagina) antigen-specific IgG and IgA responses to both HIV gp120 and MHC class I alleles, and conferred significant reduction in the plasma

viral load after a rectal challenge with simian HIV.[59] In addition to mucosal humoral immune responses, s.c. vaccination can potentially enhance mucosal CMI responses. In this regard, s.c. immunization of three- to eight-week-old calves with a BRSV immunostimulating complex (BRSV-ISCOM) vaccine induced potent lymphocyte proliferation responses concomitant with high levels of IFN-γ and IL-4 production in PBMCs as well as higher antigen-specific IgA and IgG in sera, nasal passages, and BAL fluids.[60] More significantly, in spite of the presence of variable levels of BRSV-specific maternally derived antibodies, the immunized calves were significantly protected against an aerosol BRSV challenge with significant reduction in virus titers in the upper and lower respiratory tracts.[60]

Hammerschmidt *et al.*[61] demonstrated that s.c. administration of retinoic acid to mice upregulated gut-homing molecules on activated $CD4^+$ and $CD8^+$ T cells, and triggered the generation of gut-tropic IgA^+ ASCs in the skin-draining inguinal lymph nodes. Furthermore, s.c. immunization with retinoic acid plus CT or inactivated *Salmonella typhimurium* elicited robust antigen-specific anti-CT and anti-*Salmonella* mucosal immune responses in the small intestine, and protected mice from cholera-related diarrhea and oral *Salmonella* challenge. It is important to note that some vaccines, such as inactivated influenza H5N1 vaccine, administered by s.c. route successfully protected mice against heterosubtypic

challenge with potent cross-reactive antibody responses in sera and mucosal sites (such as vagina).[62, 63] Interestingly, although i.m. immunization of mice with the B subunits of Shiga toxin type 1 and 2as a fusion protein failed to induce any fecal antibody responses, the vaccination efficiently reduced fecal bacterial shedding after oral challenge with *E. coli* O157:H7.[64] On the other hand, s.c. immunization with Tir proteins and type III secreted proteins IpaB and IpaD from *E. coli* O157:H7 failed to elicit protective mucosal immunity against subsequent pathogen challenges, although strong systemic immune responses were detected.[65-67] By using a combined s.c. and i.m. immunization strategy, rhesus macaques vaccinated with a vaccine comprising of *Chlamydia trachomatis* serover F native major outer membrane protein (MOMP) with CpG-2395 and Montanide ISA 720 VG as adjuvants developed potent systemic and mucosal humoral and CMI responses with high levels of antigen-specific IgG and IgA in plasma and mucosal secretions (vaginal washes, tears, saliva and stools),as well as enhanced lymphocyte proliferation responses and IFN-γ, TNF-α and IL-6 production by PBMCs.[68]

Intradermal immunization

Intradermal (i.d.) vaccination, developed for the original smallpox vaccine and referred as scarification at the time, was reported to induce both systemic and mucosal immune responses.[69, 70] In pigs, i.d. immunization with a commercial

inactivated *M. hyopneumoniae* whole-cell vaccine elicited robust *M. hyopneumoniae*-specific serum IgG and pulmonary IgA responses, significantly increased level of IL-10, but not IL-6, TNF-α or IFN-γ, in the BAL fluids, although the number of antigen-specific IFN-γ producing cells in PBMCs was significantly higher in the i.d. immunized pigs.[71] Mice intradermally immunized with a HPV DNA vaccine together with a CTB plasmid vector generated high antigen-specific IgA and IgG titers in cervical secretions and feces, and showed enhanced CTL activity and Th1 (IL-2 and IFN-γ) cytokine expression in spleen.[72] Interestingly, i.d. administration of a sperm-DNA vaccine to female mice elicited mainly IgG responses in sera and largely IgM and IgA responses in the vaginal wash fluid.[73] Pigs i.d. immunized with a DNA vaccine showed significant reduction of gross pathological lesions and bacterial shedding in urogenital tract after a vaginal *C. Trachomatis* challenge.[74] It has been recently shown that i.d. vaccination of mice with inactivated influenza virus using microneedles induced more robust serum and lung IgG responses, increased expression of IL-4 and IFN-γ in spleen and IL-12 in lung, and provided better protection against i.n. viral challenge than i.m. vaccination. [75] Moreover, i.d. immunization (using microneedles) of mice with IpaB and IpaD adjuvanted with double mutant *E. coli* heat labile toxin (dmLT) resulted in the local recruitment of APCs (macrophages, $CD11c^+$ dendritic cells and Langerhans cells), serum IgG responses, and

secretion of various cytokines from T cells. The vaccinated mice were protected against lethal pulmonary challenges with *S. flexneri* (70% survival) or *S. sonnei* (50% survival) although little mucosal immune responses(mucosal IgA or mucosal and systemic IgA-ASCs) were detected.[76] However, some vaccines (such as a β-galactosidase and a rotavirus DNA vaccine) administered by i.d. route failed to induce sufficient mucosal antibodies or to protect against mucosal challenge.[77-79] The observation of adverse local reactions caused by i.d. injection or scarification in some studies should be considered in the future applications of i.d. vaccination to protect against mucosal pathogens.[69]

Transcutaneous immunization

Transcutaneous immunization (TCI) is an approach of delivering the vaccine through the skin layer. Since this method requires some physical/chemical means to breach the intact skin so as to deliver the antigen/adjuvant into the epidermal layer, it is discussed in this review in the context of potential to elicit mucosal immunity although it is debatable whether TCI is truely a systemic immunization or not. Vaccination by TCI would be more acceptable by the patients as opposed to by traditional i.m., i.d., or s.c. methods, and TCI has been demonstrated to induce robust systemic and mucosal immune responses that protect the host against mucosal infection.[80-84] For example, CT- or CpG-adjuvanted chlamydial MOMP applied to the shaved skin on the back region of mice enhanced

MOMP-specific IgG and IgA responses in sera, vaginal and uterine lavage fluids, and increased IFN-γ (but not IL-4) mRNA expression in the mononuclear cells from the reproductive tract-draining caudal and lumbar lymph nodes, and protected the mice against an intravaginal *C. muridarum* challenge.[85] Moreover, an adjuvant-free, powdered, inactivated influenza vaccine placed on the shaved abdominal skin of mice elicited specific IgG and IgA responses in serum and at several mucosal sites (e.g, small intestine, saliva, vagina, and nasal passages), and effectively increased the survival rate of mice against an i.n. challenge with the influenza virus.[86].Furthermore, based on the presence of antigen-specific IgA secreting ASCs in lamina propria of small intestine and the secretion of specific IgA from *in vitro* cultured tracheal and small intestinal samples, it was suggested that the antigen-specific antibodies were locally produced at the relevant mucosal sites, rather than diffusing from sera .[86] Specific IgG and IgA to both tetanus toxoid (TT) and CT were detected in sera, saliva, vaginal lavages and fecal extracts of mice transcutaneously immunized with TT admixed with CT, with comparatively higher titers in sera, saliva and vaginal lavage as compared to in fecal pellets.[87] In another study, it was shown that TCI with CT or its B subunit (CTB)elicited more potent anti-CTB serum IgG responses and comparable specific IgA responses in serum, feces and bile, when compared to oral immunization with live vaccine strain of *Vibrio cholerae* expressing CTB.[88]

The immune responses elicited by s.c., i.d. and TCI immunization of an HIV nanoparticle vaccine were compared in mice.[89] The population of antigen-specific cytokine (IL-2 or IFN-γ or TNF-α) producing $CD4^+$ T cells in the spleen from i.d. or s.c. immunized mice were significantly higher than those from TCI mice. However, the population of poly functional T cells which produce all three cytokines (IL-2, IFN-γ and TNF-α) was highest in TCI group, and lowest in i.d. immunized group. Significantly increased antigen-specific $CD8^+$ T cells were found in blood after i.d. and TCI immunization while absent after s.c. immunization, consistent with higher population of $CD3^+CD8^+$ T cells in vaginal mucosa of TCI and i.d. vaccination when compared to s.c. vaccination. These results suggest that TCI and i.d. immunization redirected homing of antigen-specific effector/memory $CD8^+$ T cells to the vaginal mucosa. Interestingly, TCI of mice at different anatomic skin sites (back, abdomen, and ear) induce different magnitude of systemic (spleen) and mucosal (PPs) CTL responses, with the strongest CTL responses in both mucosal and systemic sites elicited by TCI on the back.[87] In contrast, TCI immunization of mice with a synthetic hexasaccharide-protein conjugate vaccine failed to induce detectable mucosal immune responses or provide any protection against oral *V. cholera* challenges despite the presence of robust serum IgG and IgA responses.[90] In a double-blind, placebo-controlled clinical trial wherein 59 randomized adults

were transcutaneously immunized with either the LT from enterotoxigenic *E. coli* (ETEC) or placebo, higher serum IgG and IgA as well as fecal IgA responses were detected in vaccinees compared to the placebo controls.[91] However, the vaccination only mitigated, but did not prevent, the infection after an oral challenge with a virulent ETEC strain.

Potential mechanisms of systemic vaccination-induced mucosal immunity

Although systemic immunization (s.c., i.m., i.d., i.p. and TCI) can induce mucosal immune responses under certain antigen and adjuvant combinations, the mechanism of this induction remains poorly understood, So far, several mechanisms have been proposed to explain the induction of mucosal antibodies after systemic immunization. Based on the relatively low number of APCs in some of the systemic tissues, it was hypothesized that an antigen first diffuses from a s.c., i.m. or i.p. immunization site to the regional draining lymph nodes, and from there is taken up by the local APCs (such as DCs, B cells, and macrophages). These APC cells then migrate to the mucosa-associated lymphoid tissue (MALT), such as PPs and nasopharynx-associated lymphoid tissue (NALT), where they activate $CD4^+$ T cells and B cells.[92, 93] On the other hand, antigen administered by i.d. or TCI can activate APCs, mainly the Langerhans cells and DCs, in the epidermis and dermis of the skin. These cells migrate to MALT and present the antigen to naïve T cells for the generation of antigen-specific T cells,

including Th1, Th2, Th17, and cytotoxic T cells.[94, 95] Alternatively, soluble or phagocytosed antigens may migrate to the MALT directly.[3]

The immunostimulatory molecules (such as those provided by adjuvants) in the vaccines increase the local recruitment, antigen processing and presentation efficiency of the APCs at the site of vaccination, promote the proliferation of antigen-specific T cells and antibody-secreting B cells, which then migrate to the distant effector sites, such as lamina propria (LP) of the gut and salivary glands [96,95, 97] Under the influence of the specialized mucosal homing and imprinting mechanisms, antibody-secreting B cells finally differentiate into plasma cells and produce specific antibodies whereas a subpopulation of the antigen-activated T cells expressed different adhesion molecules, depending on the anatomic location of the lymph nodes and differentiated as tissue-resident memory T cells (T_{RM}). Recent studies indicate that these T_{RM} cells persist in the tissue long after vaccination or the clearance of the infection for maximal and efficient control of locally invaded pathogens.[98, 99,100] In addition, mucosal antibody responses can also be induced through exudation, transcytosis, or production by the local plasma cells.

Conclusion

Based on the published literature to date, it is well recognized that the protective efficacy of a vaccine delivered by varying routes of immunization is affected by

the choice of the antigen, the antigen carrier/delivery vehicle, and the adjuvant, amongst many other factors. It is also generally acknowledged that immunization via mucosal routes, using subunit antigens, can elicit robust mucosal (and systemic) immune responses that accord protection against specific mucosal pathogens. Increasing evidence from experimental vaccine and animal model studies suggest that under some circumstances (antigen, adjuvant, delivery vehicle) systemic routes of immunization have the potential to induce immune responses in both the systemic and multiple mucosal compartments. However, it is currently unknown as to under what specific circumstances would a systemic immunization elicit a protective mucosal immune response in an animal model, or if the observations in animal models would be translated to human subjects? However, it appears that i.p. immunization generally induces non-protective mucosal (particularly the gastrointestinal) IgA responses while i.m. immunization with DNA-based vaccines is likely to induce a protective mucosal immune response including CMI. In addition, TCI appears to be another encouraging route of systemic immunization to induce protective mucosal immunity.

The results from many studies on the potential of systemic immunization to elicit protective mucosal immunity in animal models are often difficult to interpret because there is the failure to evaluate whether the protection (if seen) was due

to the mucosal immune responses elicited or could have been as a result of strong systemic responses *per se*? Part of the challenge may be that there are no appropriate animal models of disease, wherein it is clearly known that only a strong mucosal immune response would protect the vaccinated host against the specific, mucosal pathogen challenge. Moreover, observations on the presence of mucosal immune responses (such as serum and salivary IgA) in human subjects that have been immunized with a systemic vaccine were often complicated by the prior exposure to the antigens or pathogens. In spite of these limitations, the ongoing studies to date do indicate that there is the potential to develop systemic vaccination strategies that may offer an alternative approach to mucosal immunization for the elicitation of both mucosal and systemic immune responses.

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Table 1. Levels of antigen-specific IgA antibody responses at systemic and mucosal sites

Delivery route	Gastrointestinal Tract			Respiratory Tract		Reproductive Tract		Serum
	Saliva	Intestine	Feces	Upper	Lower	Vagina	Uterus	
i.p.	-(21,23,24,26) + (16,20)	++ (18,20,26)	+ (26) ++(28) +++ (27)		-(18,20,21,24,25) +(26) ++(27) +++(17)	+(18,26,28) ++(20)	+++(27)	-(27,28) +(18,23)
i.m.	+(33) ++(35)	++(47)	+(37) ++(32,47)	-(55) ++(34,35) +++(38)	-(30,38,41,50) +(36,47,49) ++(34)	+(31)		-(34) +(31,36,37) ++(47) +++(38)
s.c.	++(68)	+(59) ++(58) +++(61)	-(64,65,66) +(68)	++(60)	-(56,57,63,67)	-(62) +(68) ++(59)		-(57,62,63,66,67) ++(59,60,68) +++(61)

i.d.			-(76,77,78) +++(72)	-(79)	-(76,77) ++(69,71)	++(70,73)	+++(72)	-(76,78) +(73,77) ++(74)
TC	+(86) ++(97)	+(88) ++(86)	-(90) +(88) ++(80,81,97)	+(86)	+(83)	+(85,97) ++(86)		-(90) +(84,88,97) ++(80,82)

Levels were scored as none (-), slight (+), moderate (++), or strong (+++) based on the primary publication.

The numbers in the parenthesis refer to the citations in the References.

Table 2. Levels of antigen-specific IgG antibody responses at systemic and mucosal sites

Delivery route	Gastrointestinal Tract			Respiratory Tract		Reproductive Tract		Serum
	Saliva	Intestine	Feces	Upper	Lower	Vagina	Uterus	
i.p.	+(20,23)	++(18) +++(20)	+++(27,28)		++(18,20,27)	+(18,28) ++(20)	++(27)	+(23) ++(25) +++(16-21,24-28)
i.m.	+(33)			-(38)	++(30) +++(38)	+(31) +++(39)	+(40) +++(39)	+(36)++(30,52) +++(31-35,37-39,41)
s.c.	++(68)	+(59)	+(68)	+++(60)	++(56,57) +++(63,67)	+(62,68) ++(59)		++(61,62,68) +++(57,59,60,63-67)
i.d.			++(72)		++(76) +++(75)	+(73,76)	+++(72)	-(76,78) +(73,77) ++(74)+++(75)

	TC	++(86)	+++(86)	++(97)		+++(80)	+(85)		++(90)
Leve		+++(9		+++(80)		++(83)	+++(86,9		+++(80,81,83-85,86,8
ls		7)					7)		8,97)

were

scored as none (-), slight (+), moderate (++), or strong (+++) based on the primary publication. The numbers in the parenthesis refer to the citations in the References.

Table 3. Levels of antigen-specific IgM antibody responses at systemic and mucosal sites

Delivery route	Gastrointestinal Tract			Respiratory Tract		Reproductive Tract		Serum
	Saliva	Intestine	Feces	Upper	Lower	Vagina	Uterus	
i.p.		++(18)			+++(18)	++(18)		++(18)
i.m.					+(49)			++(34,41)
s.c.								++(61)
i.d.						++(73)		+(73) ++(69)
TC								-(80) +++(82)

Levels were scored as none (-), slight (+), moderate (++), or strong (+++) based on the primary publication.

The numbers in the parenthesis refer to the citations in the References.

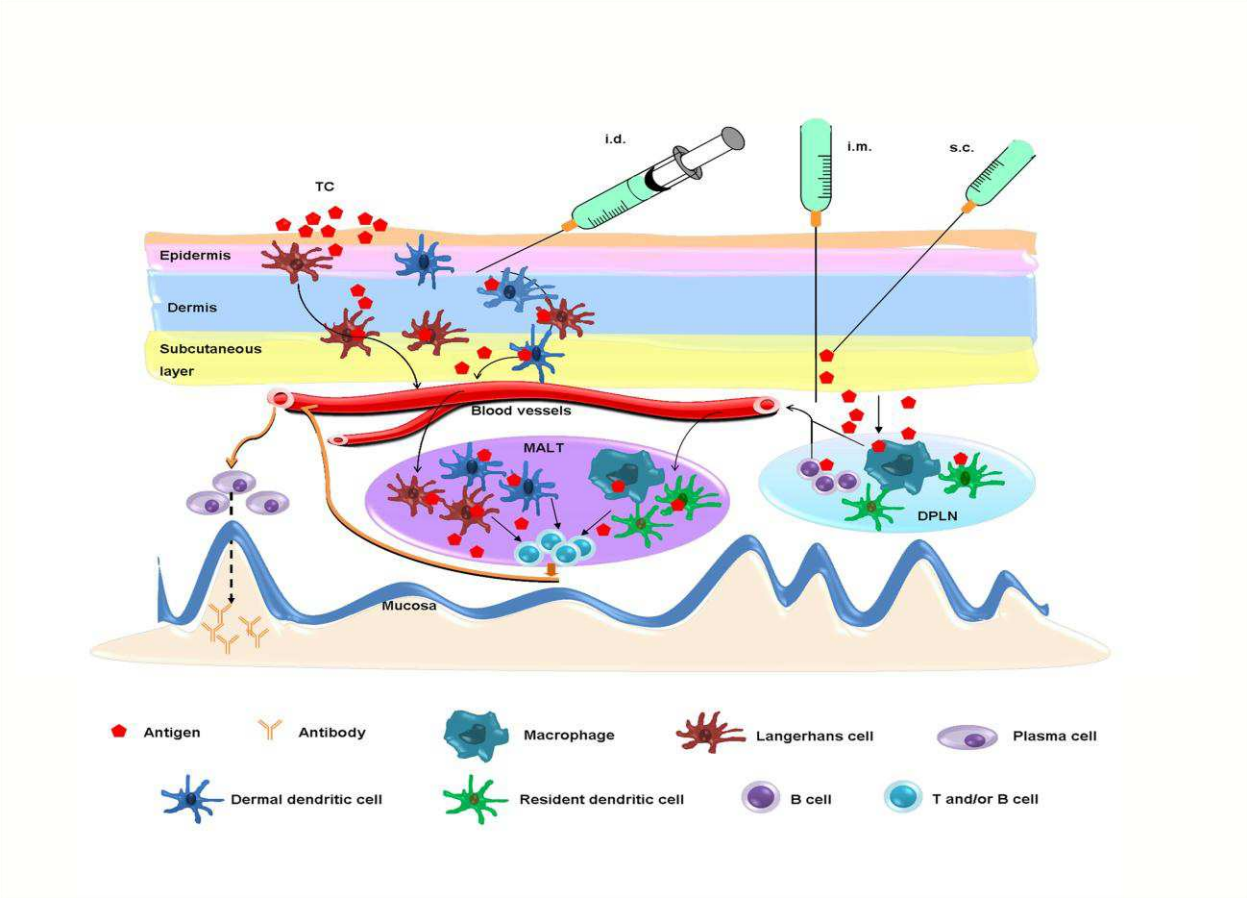

Figure 1. Potential mechanisms of systemic vaccination-induced mucosal antibody responses. Intradermal (i.d.) or transcutaneous (TC) immunization activates Langerhans cells and dermal dendritic cells in the epidermis and dermis of skin, which then migrate to the mucosa-associated lymphoid tissue (MALT) where they present the antigen to $CD4^+$ T cells and B cells. An antigen delivered by i.m. or s.c. route mainly diffuses to the draining peripheral lymph nodes (DPLN) where it activates APCs, such as B cells, dendritic cells and macrophages. Mucosal antibody responses are triggered when they reach to the MALT and present the antigen to $CD4^+$ T cells and B cells. A free antigen may migrate to MALT directly.