Chapter 55

Mucosal Vaccines: An Overview

Michael W. Russell

Departments of Microbiology/Immunology and Oral Biology, University at Buffalo, Buffalo, NY, USA

Jiri Mestecky

Departments of Microbiology and Medicine, University of Alabama, Birmingham, AL, USA; Institute of Microbiology, Czech Academy of Sciences and Department of Immunology and Microbiology, School of Medicine, Charles University, Prague, Czech Republic

Chapter Outline

Although immunization by mucosally applied vaccines has had a long history with numerous microorganisms and a variety of administration routes (see *Historical Aspects of Mucosal Immunology*), only a few such vaccines are currently used in human medicine (Table 1). Thus the enormous potential of immunity in the mucosal tissues and their associated secretory glands remains to be exploited in vaccinology. Yet it should be kept in mind that the vertebrate immune system has evolved in a close relationship with the mucosae, and it remains strategically associated with mucosal surfaces, which constitute the most important site of continuous stimulation with environmental antigens, including the microbiota, and the portal of entry for most infections. A much larger number of mucosal vaccines, however, has been developed for veterinary use, especially to be administered in the feed of farmed animals (Chapter 68).

The functional separation of the mucosal immune system from the systemic (circulatory) compartment means that different vaccination approaches must be employed to achieve effective mucosal immunity. On the other hand, the "common" mucosal immune system (CMIS), with its central inductive sites in the intestinal and respiratory tracts that provide for the dissemination of responses to remote effector sites, affords an opportunity to direct immune responses to the particular sites where protection is required. This is made feasible through a system of "homing" receptors, addressins, and chemokines that allows the imprinting of lymphocytes during their antigenic stimulation within the inductive sites with selective homing potential to different effector sites according to where they were originally induced (see Chapter 40). Thus the CMIS is not uniform, but it is amenable to selective manipulation by different mucosal vaccination strategies.

RATIONALE FOR MUCOSAL VACCINATION

There are many advantages that make mucosal vaccines an attractive alternative to those administered by systemic routes. Most importantly, protective immune responses can be induced at the relevant mucosal sites of pathogen entry by mucosal delivery of vaccines (Table 2). Numerous examples clearly demonstrate the validity of this strategy, including intranasal inactivated or attenuated influenza virus vaccines, the oral polio vaccine, and oral attenuated *Salmonella* Typhi and *Vibrio cholerae* vaccines, among many others currently under development against various enteric and respiratory infections (Chapters 56–59). Furthermore, in epidemics of mucosally transmitted infections, mass immunization by mucosal routes is likely to be more practicable and less expensive than immunization by systemic routes. The reduced cost of mucosal vaccines is realized at both production and delivery levels. The purity of mucosally delivered vaccines, including endotoxin contamination, is less critical than for injectable vaccines. Some mucosal vaccines (e.g., plant-expressed vaccines such as Mucorice®; Chapter 65) do not require storage at low temperature and remain stable and fully immunogenic when kept at ambient temperatures for extended periods. Finally, mucosal vaccine delivery does not require sterile syringes and needles or personnel trained in their use and disposal,

Mucosal Immunology. http://dx.doi.org/10.1016/B978-0-12-415847-4.00055-0

TABLE 1 Existing Mucosal Vaccines for Human Application

Infection	Vaccine	Route	Comments
Poliomyelitis	Oral polio vaccine (Sabin)	Oral	3 attenuated serotypes
Salmonella Typhi	Ty21a (Vivotyf®)	Oral	Live attenuated *S.* Typhi
Cholera	Dukoral® CVD103-HgR	Oral Oral	Killed *Vibrio cholerae* plus CTB Live attenuated *V. cholerae*
Influenza	HA plus LT Flumist®	Intranasal Intranasal	Withdrawn Attenuated virus
Rotavirus	Rotashield® Rotarix® RotaTeq®	Oral Oral Oral	Withdrawn Attenuated virus, monovalent Attenuated virus, pentavalent
Adenovirus	Adenovirus	Oral	Live adenovirus types 4 and 7, military use only

Abbreviations: CTB, cholera toxin B subunit; HA, hemagglutinin; LT, *Escherichia coli* heat-labile enterotoxin.

TABLE 2 Routes, Advantages, and Disadvantages of Mucosal Vaccines

Route	In Use?	Advantages	Disadvantages
Oral (enteric)	Yes	Most convenient CMIS dissemination Applicable to GI, also oral, genital? infections	Destruction by gastric acid and digestive enzymes Needs delivery system and/or adjuvant
Intranasal	Yes	Simple CMIS dissemination Applicable to URT, also oral, LRT, genital infections	May need spray device Risk of retrograde neural uptake in olfactory nerve (some adjuvants and antigens)
Sublingual	No[a]	Simple Applicable to URT, also oral, LRT, genital? infections Avoids complications of intranasal	None known Mechanisms of dissemination uncertain Target tissues uncertain
Rectal	No[a]	Applicable to lower GI, also genital infections	Low acceptability Limited to lower GI, genital tracts
Intravaginal	No[a]	Applicable to female genital tract	Only applicable to female

Abbreviations: URT, upper respiratory tract; LRT, lower respiratory tract; GI, gastrointestinal.
[a]*Experimental use only, mainly in animals (few in humans).*

although spray devices or other applicators may be needed for intranasal and other routes of administration.

Reluctance towards broader implementation of mucosal vaccination probably owes much to ingrained attitudes among the public, who invariably associate vaccines with hypodermic syringes, and to limited acceptance by the medical profession and large pharmaceutical companies, which seem to prefer the proven technology of injecting nonviable vaccines, although these must meet very high safety profiles before licensure. In addition, many parents already hesitate to subject their young children to repeated needle-sticks. Nevertheless, there are some disadvantages for mucosal vaccines (Table 2), particularly the uncertainty of dose delivery of mucosally administered antigens. Because of the well-demonstrated limited uptake of intact protein and polysaccharide antigens at mucosal membranes, as well as the potential for degradation, especially in the gastrointestinal tract, significantly higher doses of vaccine antigens must be administered to induce measurable responses. To overcome such difficulties, various particulate antigen delivery systems have been explored, as described in Chapter 63. In addition to the prevention of enzymatic degradation of antigen, formulation in various types of particles increases uptake. Furthermore, immuno-enhancing adjuvants and cytokines can be incorporated along with molecules that promote uptake by M cells in the follicle-associated epithelium covering the inductive sites (see below).

Live bacterial and viral vectors (Chapters 64 and 66) have been extensively explored for mucosal immunization in animal experiments, with the objective of delivering expressed protein antigens to the mucosal inductive tissues. Problems encountered in these approaches include low expression levels of the cloned antigens and improper folding of the polypeptide backbone and lack of appropriate glycosylation, both of which may result in irrelevant immune responses. Moreover, repeated use of the same microbial vector can induce dominant immune responses to the vector rather than the desired antigen, thereby precluding multiple use of the same vector, either for boosting immune responses or for delivering different vaccines. Preexisting humoral immunity to some common microbial

vectors, such as gram-negative enteric bacteria, or adeno- and influenza viruses, is likely to limit the effectiveness of mucosal vaccines based on them, even for priming. On the other hand, the use of commensal bacteria as vectors, which have coevolved with their hosts in a state of mutual coexistence, might result in hyporesponsiveness or even the induction of tolerance instead of active immunity, unless immunostimulatory molecules are also included, although this might then result in undesirable responses against the commensal vector.

As an alternative to intact microbial vectors, which (particularly in the case of viral vectors) may work by delivering DNA or RNA encoding vaccine antigens into host cells, direct immunization with DNA or RNA has also been explored in the context of mucosal vaccination. For the most part, DNA vaccination efforts have focused on the use of systemic delivery methods, but some studies have been performed on mucosal delivery, as discussed in Chapter 67.

Despite extensive efforts over many years to develop a vaccine against HIV (Chapter 60), only one (systemically administered) vaccine has displayed measurable (~30%) protection. As in almost all other trials, mucosal immune responses were not evaluated. Given that the great majority of HIV infections worldwide are acquired through mucosal transmission, this is most regrettable, as it is difficult to determine the correlates of protection if only systemic immune responses are evaluated, while ignoring the possibility of protective responses at the mucosal sites of infection. Future vaccination efforts should address this unfortunate omission. Mucosal tissues and fluids can be sampled without difficulty from primates, and are capable of providing detailed information concerning both cellular and humoral responses in relevant mucosal organs.

It is interesting, although not well appreciated, that the search for a vaccine against dental caries (thought to be the world's most frequent bacterial infectious disease) for many years led the field of oral immunization. Indeed, the first demonstration in humans that oral immunization could induce specific IgA antibodies in saliva and other secretions was carried out using *Streptococcus mutans*, the principal causative agent of caries (Mestecky et al., 1978). Considerable progress was achieved (Chapter 69), revealing much about the workings of the mucosal immune system. A more challenging goal for oral health has been to develop a vaccine against periodontal disease, an infection-driven chronic inflammatory disease of complex etiology, also discussed in Chapter 69.

MUCOSAL ADJUVANTS

One of the key issues in all vaccine development is the identification of appropriate adjuvants to enhance the desired aspects of the immune response against the vaccine antigens. Adjuvants for mucosal application involve different requirements from those for parenteral use, but they also afford greater opportunities for discovery by exploiting the growing understanding of the mechanisms whereby mucosal pathogens and commensals interact with the immune system.

A classic example is cholera toxin (CT), whose mucosal adjuvant properties were revealed by its ability to break tolerance (Elson and Ealding, 1984). A huge amount of literature has since accumulated, demonstrating the potent adjuvanticity of CT and related heat-labile enterotoxins such as *Escherichia coli* labile toxin (LT) and its subtypes (Chapter 61). A major concern for human application has been to separate the toxicity of these molecules from their adjuvant properties, whether by targeted mutations in the enzymatically active site of the A subunit, selection of different toxin types (or mutants) that have different ganglioside (receptor)-binding B subunits, or use of nontoxic B subunits alone or coupled to the vaccine antigens. The route of application has generated interest and concern with the finding that CT and LT can undergo retrograde migration along neurons, potentially reaching the brain via the olfactory nerve if administered intranasally, and hence giving rise to neurological pathology. An intranasal influenza vaccine containing a low dose of LT as an adjuvant was introduced in Switzerland, but it was withdrawn amid suspicions of causing Bell's palsy in some recipients (Mutsch et al., 2004). Given the utility of intranasal vaccination for eliciting protection, not only in the upper respiratory tract, mouth, and associated tissues, but also in the genital tract, comprehension of the mechanisms of neural uptake and how it can be obviated becomes important. As an alternative route, sublingual vaccination has been demonstrated to achieve similar responses that are disseminated to the same remote effector sites (Czerkinsky et al., 2011) but without the risk of retrograde transmission to the brain. Other bacterial toxins and plant lectins that bind glycoconjugates on mucosal cell surfaces have also been shown to have immunomodulatory properties (Lavelle et al., 2001), though none of these has been investigated as extensively as the heat-labile enterotoxins.

The discovery that many adjuvant-active materials are ligands for various pattern-recognition receptors, including the Toll-like receptors (TLR), has stimulated interest in the mechanisms of adjuvant action, mostly in the context of systemic immunization. However, among these, bacterial lipopolysaccharide and its derivative, monophosphoryl lipid A, and so-called "CpG" oligodeoxynucleotides, which are ligands for TLR4 and TLR9, respectively, have been examined as mucosal adjuvants (Chapters 61 and 62). Given its occurrence in many enteric bacteria, flagellin, a ligand for TLR5 that is expressed on mucosal epithelial cells, has also been considered as a candidate mucosal adjuvant.

Functionally related to adjuvants are delivery systems for mucosal vaccines. Because it is known that microparticulate

antigens are readily taken up by M cells in the follicle-associated epithelium covering mucosal inductive sites, a large variety of particulate vaccine formulations has been described in the scientific literature and patent applications. These include biodegradable polymer microparticles, liposomes and similar lipid-based vesicles, "immunostimulating complexes" (ISCOMs) formed with plant-derived saponins (such as Quil A), and even emulsions and bioadhesive polymers that enable vaccine preparations to adhere to mucosal membranes (see Chapter 63). Such formulations also allow the sustained release of vaccine antigens and provide some protection against degradation by gastric acid and intestinal enzymes. Scope exists also to target microparticles for uptake by incorporating into their surfaces molecular ligands for receptors on epithelial (including M) cells or even the antigen-presenting cells within the tissues.

DURATION OF RESPONSES: MUCOSAL MEMORY

For mucosal vaccines to be effective, it is desirable that secretory IgA (S-IgA) antibody responses should persist, or at least be very rapidly recallable in the face of pathogenic attack, in order to forestall infection at the mucosal surface. However, it appears that S-IgA antibodies do not always persist for long after the removal of the inducing antigenic stimulus. A paper addressed this important issue (Hapfelmeier et al., 2010) (see also Chapter 23) by demonstrating that, in gnotobiotic mice reversibly colonized with mutant *E. coli*, intestinal IgA antibody responses persisted in the absence of competing stimuli but were progressively attenuated when the mice were also colonized by other intestinal microbes. Earlier, it had been shown that enteric immunization of mice with the potent immunogen and adjuvant, CT, induced memory cells in the gut lamina propria that could be rapidly recalled, and intestinal memory to CT can persist for the lifetime of mice (Harrod et al., 2001; Lycke and Holmgren, 1986). It is known that long-lived plasma cells survive in the bone marrow, and a substantial proportion of these produce IgA (Alley et al., 1982), but this is confined to the circulatory compartment and not destined for mucosal secretions. On the other hand, mucosal IgA-secreting cells can be found in the circulation (Kutteh et al., 1980; Mei et al., 2009), and aerosolized antigen can recall IgA-, as well as IgG- and IgE-secreting plasma cells to the lungs (Luger et al., 2009). However, these responses declined after cessation of antigen exposure, and long-term plasma cells remained only in the bone marrow. Field experience with the oral cholera vaccine (Dukoral®), for example, shows that protective immunity wanes after ~3 years (Clemens et al., 1990). Conversely, the oral polio vaccine generates long-term immunity, possibly because as an attenuated live viral vaccine it persists and continually restimulates T and B cells (Ogra et al., 1968). Thus a key issue is to comprehend the parameters of memory within the mucosal immune system, what it takes to establish long-term memory T and B cells, where such cells reside, and how they can be rapidly recalled to mucosal surfaces by the reappearance of corresponding antigen. Otherwise, vaccination strategies that provide long-term stimulation, or periodic revaccination, may be required to maintain protective immunity at mucosal surfaces.

MUCOSAL TOLERANCE

Extended exposure to large doses of proteins by oral or nasal routes results in markedly diminished or even totally abrogated cellular and humoral immune responses in the systemic compartment upon subsequent immunization with the same antigen, i.e., mucosal tolerance (see *Historical Aspects of Mucosal Immunology* and Chapter 41). The term "oral tolerance" originally meant the suppression of systemic immune responsiveness induced by the prior oral administration of the same antigen (Chase, 1946). The term has been expanded to "mucosal tolerance" because the same phenomenon of suppression of a subsequent systemic immune response can be induced intranasally, or possibly even at genital surfaces (Black et al., 2000; Waldo et al., 1994). However, much confusion has arisen in the literature by the application of the term to any state of hyporesponsiveness at mucosal surfaces. While it may be true that the default mode of immune responsiveness at many mucosal surfaces is one of muted or even absent responses, that is not the same phenomenon. Immune responses can fail to occur for a variety of reasons, including inadequate antigenic stimulation, possibly as a result of low uptake or destruction of the antigen, genetically determined inability to respond, or avoidance of or interference with host responses by microbes. These may be quite different from the active suppression of responses by the induction of endogenous regulatory mechanisms. Comprehension of the regulation of mucosal immune responses and of the cell types involved has grown enormously in recent years, as documented in several new chapters in this edition (see Section B).

Although there are pronounced species differences, mucosal tolerance has been explored in numerous experiments performed in rodents and also in humans. In the latter, mucosal tolerance induced by enteric or intranasal administration of a neoantigen, such as keyhole limpet hemocyanin, was manifested by decreased T cell responses (in vitro proliferation, cytokine production, and in vivo decreased delayed-type hypersensitivity reaction). In contrast, B cell responses were primed rather than suppressed, as revealed by heightened antibody responses in serum and external secretions upon systemic immunization (Husby et al., 1994). Potential induction of mucosal tolerance has been considered an obstacle in mucosal vaccinology (Mestecky et al., 2007). However, several studies indicate that mucosal

tolerance may be a barrier only when T cell responses, including cytotoxic T cells, are critical in the mechanisms of protection.

On the other hand, exploitation of mucosal tolerance has been proposed as a means of suppressing undesirable immune responses. For example, can atopic allergies or autoimmune diseases be ameliorated after their onset by the oral administration of the offending antigens? Despite numerous efforts, it has generally proven very difficult to tolerize *after* an active immune response has been induced, especially in humans. In contrast, however, suppression of previously induced responses has been demonstrated in mice by the oral administration of antigen coupled to the recombinant B subunit of CT with the elicitation of regulatory T cells (Sun et al., 2006, 1994). Amelioration of autoimmune uveitis and experimental autoimmune encephalitis has been achieved in murine models using this strategy (Phipps et al., 2003; Sun et al., 1996).

In studies performed in humans using keyhole limpet hemocyanin as a neoantigen, oral or nasal immunization *primed* but did *not tolerize* for humoral immune responses (Husby et al., 1994; Waldo et al., 1994; Kraus et al., 2004). Furthermore, in conformity with early reports (Chase, 1946), humans and animals already sensitized cannot subsequently be tolerized by ingestion of large doses of the same antigen (Moldoveanu et al., 2004). Thus the danger of inducing mucosal tolerance with decreased T cell responses, including cytotoxic T cells, by mucosal vaccination may be of greater concern in immunologically naïve individuals, i.e., those not previously exposed to the target antigens (such as HIV) than in those who have preexisting systemic or mucosal immunity (Mestecky et al., 2007). The temporal sequence of exposure and immunization is important: initial systemic exposure followed by mucosal vaccination should obviate the induction of mucosal tolerance that leads to diminished T cell responses. In this context, it should be noted that most currently available vaccines, with a few possible exceptions, exert their protective effects by inducing *antibody* responses in serum and the external secretions.

ROUTES OF MUCOSAL VACCINE ADMINISTRATION

To induce immune responses at desired locations, experimental vaccine antigens have been administered to mucosal surfaces of the conjunctival sac, oral mucosa, tonsils, sublingual space, nasopharynx, nasal cavity, intestinal tract (including the rectum), and female genital tract. In several studies, antigens have been injected or instilled directly into external secretory glands such as the lacrimal, salivary, or lactating mammary glands (for review see Mestecky, 1987). Not surprisingly, the magnitude and quality of ensuing immune responses can be highly variable due to many factors. These include the surface area of antigen exposure, type of epithelia (squamous stratified or single layer), presence of organized lymphoepithelial structures with M cells as inductive sites (e.g., Peyer's patches), phenotypes and functions of cells involved in antigen uptake, processing, presentation (epithelial cells, dendritic cells, macrophages, B cells; see Chapters 25-29), and the presence of competing food or microbial antigens, all of which impact mucosal vaccines, particularly in the intestinal tract. The site of mucosal vaccination also plays a decisive role in the breadth of the responses, which may be restricted only to the site of antigen exposure, disseminated to other, anatomically remote mucosal sites, or include pronounced systemic responses in parallel with the mucosal responses. Almost exclusively local, humoral responses of low magnitude have been induced by immunization in the conjunctival sac, buccal mucosa, or vagina. In sharp contrast, peroral (enteric) or nasal immunization induces local, as well as disseminated, mucosal immune responses due to the presence of lymphoepithelial inductive sites, which supply remote mucosal effector tissues with the precursors of antigen-specific, Ig-isotype-committed B cells. Most interestingly, intranasal or sublingual immunization also induces immune responses in the female genital tract as well as systemically. Such responses are mediated by the selective distribution or "homing" of cells from inductive to effector sites through the CMIS (see Chapters 1 and 40). However, the receptors and mechanisms involved in homing to mucosae, other than the gastrointestinal tract, are incompletely understood.

In addition to mucosal tracts, antigen-specific immune responses are also induced in external secretory glands (e.g., lacrimal, salivary, and lactating mammary glands) that are not directly stimulated by mucosally or systemically administered antigens. Direct local injection of antigens, with or without adjuvants, into salivary or mammary glands induces strong local inflammation and humoral responses dominated by IgG and low IgA antibodies (Mestecky, 1987). Oral or nasal immunization results in the induction of antibodies in milk (mainly IgA in humans, but IgG in pigs, cows, horses, and other species) to provide essential passive immune protection to the offspring (see Chapters 116 and 117). Similarly, induction of antigen-specific S-IgA antibodies in tears and saliva can be achieved by ingestion, inhalation, sublingual, or even rectal immunization through the CMIS. However, subsequent local application of antigens at the relevant mucosal tissue (e.g., female genital tract) may further accentuate local responses.

The role of human pharyngeal lymphoid tissues (Waldeyer's ring) as inductive sites remains controversial (see Chapter 103). In some studies, tonsillectomized children responded poorly to orally administered live poliovirus vaccine (Ogra, 1971), but direct injection of antigen into a single tonsil did not induce vigorous disseminated mucosal immune responses (Quiding-Järbrink et al., 1995). The

lactating mammary gland has been considered as an organ whose IgA-producing cells are also derived from tonsillar precursors (Brandtzaeg, 1983). However, the proportion of IgA1 to IgA2 and the spectrum of antigen-specific antibodies resemble those of intestinal secretions (Ladjeva et al., 1989). Sublingual immunization has been shown to be effective in inducing S-IgA and cytotoxic T cell responses that distribute in a similar pattern to those induced by intranasal immunization (Czerkinsky et al., 2011). Rectal immunization using bacterial and viral antigens has been examined in several studies performed in animals and humans (Crowley-Nowick et al., 1997; Kantele et al., 1998; Kutteh et al., 2001), prompted by the well-documented presence of rectal lymphoepithelial tissues (rectal tonsils), which are structurally similar to Peyer's patches in the small intestine. Because of its anatomical and immunological proximity, rectal immunization has been considered for inducing mucosal responses in the female genital tract.

It should not be overlooked that several parenterally administered vaccines are effective against mucosal infections (Robbins et al., 1995) (and Chapter 70). Notably, these include vaccines against respiratory tract infections such as *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae* type b, and influenza virus, enteric (*S.* Typhi) and genital (papillomavirus) infections, as well as mucosally acquired systemic infections such as measles, mumps, and varicella viruses. Of these, the pneumococcus, meningococcus, and *H. influenzae* are carried harmlessly in the human nasopharynx but cause disease by invading systemically or (in the case of pneumococci) by descending into the lungs where systemically derived immune effectors, including IgG, are dominant. Interestingly, however, it is well documented that nasopharyngeal carriage of *H. influenzae* type b has declined dramatically since the introduction of the polysaccharide conjugate vaccine, a finding that is possibly explained by the fact that nasal secretions contain significant levels of plasma-derived IgG in addition to S-IgA. This possibly also explains the effectiveness of injectable influenza vaccines, although interestingly, intranasal vaccines have recently been introduced that appear to be equally effective. With respect to the genital tract, only vaccines against human papillomavirus have been successfully developed, and they too appear to work by inducing virus-neutralizing IgG antibodies. However, in both female and male tracts, IgG is the dominant Ig isotype, mostly derived from the circulation (see Chapters 108, 109, and 112). Although injectable vaccines were developed against enteric infections, including killed whole-cell vaccines against *S.* Typhi and *V. cholerae*, these were of very limited effectiveness and have now been withdrawn. The current injectable typhoid vaccine consists of the Vi polysaccharide capsular antigen, but it should be recalled that *S.* Typhi is an invasive bacterium that ultimately resides intracellularly within macrophages. Oral vaccines against typhoid, cholera, and rotavirus have now been developed (Table 1), and several current efforts to generate vaccines against other enteric infections exploit oral administration (Chapters 56 and 57).

Passive immunization, which for all practical purposes in humans means the administration of preformed antibodies, is directly applicable to the protection of mucosal surfaces, without entailing the adverse effects that arose from the early application of "serum therapy". Advantages include the immediate availability of protection, although duration is inevitably limited in the absence of continuous Ig production. Given that colostrum and milk are natural sources of passive immunity for neonates, artificial passive immunization is particularly applicable to the gastrointestinal tract, although not necessarily limited to it. The range of technologies now available for tailor-making specific antibodies, including those of defined isotype and desirable physiological properties, as well as strategies for their production in (for example) cows' milk, eggs, or genetically engineered plants, are discussed in Chapter 71. In addition to the gut, applications include pathogens of the oral cavity, lungs, and female reproductive tract.

Studies on various routes and strategies of mucosal immunization have spawned a set of derivative names for the inductive sites proposed to be activated, based on the concept of the mucosa-associated lymphoid tissue (MALT). Few of these tissues, however, meet the criteria that define MALT, i.e., an organized follicular lymphoid tissue with distinct B and T cell-rich zones, surmounted by a follicle-associated epithelium containing M cells, and functionally capable of disseminating antigen-activated T and B cells to other mucosal effector sites (Brandtzaeg et al., 2008). Two main sets of MALT fit the definition: gut-associated lymphoid tissue, represented by Peyer's patches in the small intestine, similar follicles in the large intestine and the appendix and sacculus rotundus (in certain species), and other isolated lymphoid follicles found in the intestine; and the nasopharynx-associated lymphoid tissue represented by Waldeyer's ring (tonsils and adenoids) in humans and analogous tissue adjacent to the nasopharyngeal tube in rats and mice. Equivalent bronchus-associated lymphoid tissue occurs in certain animals but is not normally present in adult humans. Organized inductive site tissue has also been described in the conjunctiva and lacrimal ducts, where it can generate local ocular responses (see Chapter 99). It is noteworthy that similar tissues are absent from the reproductive tract, although hormonally dependent lymphoid aggregates have been described and characterized in the endometrium of rodents, cattle, and humans (see Chapter 108). However, these aggregates, which may be induced by certain infections, have a different cellular composition not at all resembling MALT, and their functional significance remains unclear. Nevertheless, immune responses can be induced by immunization of the female reproductive tract,

probably by stimulating local populations of dendritic cells, but the responses are usually strictly local and not disseminated to remote mucosal effector sites. Strategies and routes of mucosal vaccination need to take into account the distribution of these inductive site tissues, their capacity for disseminating responses to the desired target effector sites, and the types of immune cells induced in different sites.

THE FUTURE OF MUCOSAL VACCINATION

Due to its demonstrable advantages, mucosal immunization is gaining increased acceptance in current human and veterinary vaccinology. Several efforts by commercial and academic institutions are in progress to replace injectable vaccines with effective mucosal analogs. In addition to available human mucosal vaccines, several veterinary mucosal vaccines have been introduced (e.g., baited vaccines against rabies in wildlife and vaccines given as components of feed to cattle, poultry, and farmed fish, etc.) (Chapter 68).

Other developments to improve mucosal vaccines that can be foreseen in the near future include strategies for quantitatively assured vaccine antigen uptake, development of novel mucosal adjuvants and antigen-delivery systems suitable for use in humans, and exploitation of edible plant-produced vaccines. All of these can be expected to make mucosal vaccines not only more effective, but also more widely accepted in standard medical practice for the control of infectious disease.

REFERENCES

Alley, C.D., Nash, G.S., MacDermott, R.P., 1982. Marked in vitro spontaneous secretion of IgA by human rib bone marrow mononuclear cells. J. Immunol. 128, 2604–2608.

Black, C.A., Rohan, L.C., Cost, M., Watkins, S.C., Draviam, R., Alber, S., Edwards, R.P., 2000. Vaginal mucosa serves as an inductive site for tolerance. J. Immunol. 165, 5077–5083.

Brandtzaeg, P., 1983. The secretory immune system of lactating human mammary glands compared with other exocrine organs. Ann. N. Y. Acad. Sci. 409, 353–381.

Brandtzaeg, P., Kiyono, H., Pabst, R., Russell, M.W., 2008. Terminology: nomenclature of mucosa-associated lymphoid tissue. Mucosal Immunol. 1, 31–37.

Chase, M.W., 1946. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proc. Soc. Exp. Biol. Med. 61, 257–259.

Clemens, J.D., Sack, D.A., Harris, J.R., Van Loon, F., Chakraborty, J., Ahmed, F., Rao, M.R., Khan, M.R., Yunus, M., Huda, N., Stanton, B.F., Kay, E., Eeckels, R., Walter, S., Svennerholm, A.M., Holmgren, J., 1990. Field trial of oral cholera vaccines in Bangladesh: results from three-year follow-up. Lancet 335, 270–273.

Crowley-Nowick, P.A., Bell, M.C., Brockwell, R., Edwards, R.P., Chen, S., Partridge, E.E., Mestecky, J., 1997. Rectal immunization for induction of specific antibody in the genital tract of women. J. Clin. Immunol. 17, 370–379.

Czerkinsky, C., Cuburu, N., Kweon, M.N., Anjuere, F., Holmgren, J., 2011. Sublingual vaccination. Hum. Vaccines 7, 110–114.

Elson, C.O., Ealding, W., 1984. Cholera toxin feeding did not induce oral tolerance in mice and abrogated oral tolerance to an unrelated protein antigen. J. Immunol. 133, 2892–2897.

Hapfelmeier, S., Lawson, M.A., Slack, E., Kirundi, J.K., Stoel, M., Heikenwalder, M., Cahenzli, J., Velykoredko, Y., Balmer, M.L., Endt, K., Geuking, M.B., Curtiss, R., McCoy, K.D., Macpherson, A.J., 2010. Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. Science 328, 1705–1709.

Harrod, T., Martin, M., Russell, M.W., 2001. Long-term persistence and recall of immune responses in aged mice after mucosal immunization. Oral Microbiol. Immunol. 16, 170–177.

Husby, S., Mestecky, J., Moldoveanu, Z., Holland, S., Elson, C.O., 1994. Oral tolerance in humans. T cell but not B cell tolerance after antigen feeding. J. Immunol. 152, 4663–4670.

Kantele, A., Häkkinen, M., Moldoveanu, Z., Lu, A., Savilahti, E., Alvarez, R.D., Michalek, S., Mestecky, J., 1998. Differences in immune responses induced by oral and rectal immunizations with *Salmonella typhi* Ty21a: Evidence for compartmentalization within the common mucosal immune system in humans. Infect. Immun. 66, 5630–5635.

Kraus, T.A., Toy, L., Chan, L., Childs, J., Mayer, L., 2004. Failure to induce oral tolerance to a soluble protein in patients with inflammatory bowel disease. Gastroenterology 126, 1771–1778.

Kutteh, W.A., Koopman, W.H., Conley, M.E., Egan, M.L., Mestecky, J., 1980. Production of predominantly polymeric IgA by human peripheral blood lymphocytes stimulated in vitro with mitogens. J. Exp. Med. 152, 1424–1429.

Kutteh, W.H., Kantele, A., Moldoveanu, Z., Crowley-Nowick, P.A., Mestecky, J., 2001. Induction of specific immune responses in the genital tract of women after oral or rectal immunization and rectal boosting with *Salmonella typhi* Ty21a vaccine. J. Reprod. Immunol. 52, 61–75.

Ladjeva, I., Peterman, J.H., Mestecky, J., 1989. IgA subclasses of human colostral antibodies specific for microbial and food antigens. Clin. Exp. Immunol. 78, 85–90.

Lavelle, E.C., Grant, G., Pusztai, A., Pfüller, U., O'Hagan, D.T., 2001. The identification of plant lectins with mucosal adjuvant activity. Immunology 102, 77–86.

Luger, E.O., Fokuhl, V., Wegmann, M., Abram, M., Tillack, K., Achatz, G., Manz, R.A., Worm, M., Radbruch, A., Renz, H., 2009. Induction of long-lived allergen-specific plasma cells by mucosal allergen challenge. J. Allergy Clin. Immunol. 124, 819–826.

Lycke, N., Holmgren, J., 1986. Intestinal mucosal memory and presence of memory cells in lamina propria and Peyer's patches in mice 2 years after oral immunization with cholera toxin. Scand. J. Immunol. 23, 611–616.

Mei, H.E., Yoshida, T., Sime, W., Hiepe, F., Thiele, K., Manz, R.A., Radbruch, A., Dörner, T., 2009. Blood-borne human plasma cells in steady state are derived from mucosal immune responses. Blood 113, 2461–2469.

Mestecky, J., 1987. The common mucosal immune system and current strategies for induction of immune response in external secretions. J. Clin. Immunol. 7, 265–276.

Mestecky, J., McGhee, J.R., Arnold, R.R., Michalek, S.M., Prince, S.J., Babb, J.L., 1978. Selective induction of an immune response in human external secretions by ingestion of bacterial antigen. J. Clin. Invest. 61, 731–737.

Mestecky, J., Russell, M.W., Elson, C.O., 2007. Perspectives on mucosal vaccines: is oral tolerance a barrier? J. Immunol. 179, 5633–5638.

Moldoveanu, Z., Oliver, F., Mestecky, J., Elson, C.O., 2004. Oral tolerance in humans: failure to suppress an existing immune response by oral antigen administration. Ann. N. Y. Acad. Sci. 1029, 299–309.

Mutsch, M., Zhou, W., Rhodes, P., Bopp, M., Chen, R.T., Linder, T., Spyr, C., Steffen, R., 2004. Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. New. Engl. J. Med. 350, 896–903.

Ogra, P.L., 1971. Effect of tonsillectomy and adenoidectomy on nasopharyngeal antibody response to poliovirus. New. Engl. J. Med. 284, 59–64.

Ogra, P.L., Karzon, D.T., Righthand, F., MacGillivray, M., 1968. Immunoglobulin response in serum and secretions after immunization with live and inactivated poliovaccine and natural infection. New. Engl. J. Med. 279, 893–900.

Phipps, P.A., Stanford, M.R., Sun, J.B., Xiao, B.G., Holmgren, J., Shinnick, T., Hasan, A., Mizushima, Y., Lehner, T., 2003. Prevention of mucosally induced uveitis with a HSP60-derived peptide linked to cholera toxin B subunit. Eur. J. Immunol. 33, 224–232.

Quiding-Järbrink, M., Granström, G., Nordström, I., Holmgren, J., Czerkinsky, C., 1995. Induction of compartmentalized B-cell responses in human tonsils. Infect. Immun. 63, 853–857.

Robbins, J.B., Schneerson, R., Szu, S.C., 1995. Perspective: hypothesis: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. J. Infect. Dis. 171, 1387–1398.

Sun, J.-B., Raghavan, S., Sjöling, Å., Lundin, S., Holmgren, J., 2006. Oral tolerance induction with antigen conjugated to cholera toxin B subunit generates both $Foxp3^{+}CD25^{+}$ and $Foxp3^{-}CD25^{-}$ $CD4^{+}$ regulatory T cells. J. Immunol. 177, 7634–7644.

Sun, J.B., Holmgren, J., Czerkinsky, C., 1994. Cholera toxin B subunit: an efficient transmucosal carrier-delivery system for induction of peripheral immunological tolerance. Proc. Natl. Acad. Sci. U.S.A. 91, 10795–10799.

Sun, J.B., Rask, C., Olsson, T., Holmgren, J., Czerkinsky, C., 1996. Treatment of experimental autoimmune encephalomyelitis by feeding myelin basic protein conjugated to cholera toxin B subunit. Proc. Natl. Acad. Sci. U.S.A. 93, 7196–7201.

Waldo, F.B., van den Wall Bake, A.W.L., Mestecky, J., Husby, S., 1994. Suppression of the immune response by nasal immunization. Clin. Immunol. Immunopathol. 72, 30–34.