MUCOSAL IMMUNIZATION

The mucosal route of administration could be a viable solution for the billions who live in conditions of extreme poverty.

Here I stay, with words and villages and roads that await me again, that knock on my door with starry hands.'

_I Am Going to Live_
Pablo Neruda

The Market
Manuel Mendive
Oil on canvas; 152 × 203 cm
The advantages of the oral route for immunization are undeniable. It is the physiological route for voluntary entry of substances into the organism. It does not require specialized personnel, and the regulations indicate that the required manufacturing facilities are less expensive, reducing the cost of vaccine production.

‘... I see in the misery, the naked foot of a child.’

The Keys of the City
Jacques Prevert

Cosmogonía
Roberto Alvarez Ríos
Oil on canvas; 140.5 × 200 cm
Collection of the National Museum of Fine Arts, La Habana, Cuba
MUCOSAL IMMUNITY:
IMMUNITY WHERE IT IS MOST NEEDED

Most pathogens enter the body and/or establish infection in the mucosal tissues such as those of the respiratory, the gastrointestinal and the urogenital tracts. Hence, induction of protective immunity at the mucosal surfaces is fundamentally important to counter mucosally transmitted pathogens.

Mucosal surfaces are equipped with innate and acquired immune defense mechanisms that cooperate in protecting mucosal tissues from invading pathogens (1). Mucosal tissues are separated from the external environment by epithelial barriers organized as a single layer (in the nasal and intestinal mucosa) or stratified layers (in the oral cavity and vagina) of epithelium. Epithelial barriers play a pivotal role in containing pathogens both mechanically and through production of innate defense molecules such as various mucins and anti-microbial factors. Not only as a physiochemical barrier, epithelial cells can also sense and respond to microbial components through various pattern recognition receptors thus producing cytokines and chemokines that influence development of protective innate and adaptive immune responses.

The mucosal immune system contains two functionally distinct types of tissues: the inductive site where B cells and T cells meet antigen (Ag) and become activated; and the effector site where activated lymphocytes exert their effector functions (2). Mucosal immune responses following pathogen encounter or mucosal immunization are kicked off in the mucosa-associated lymphoid tissue (MALT) structures such as the Peyer’s patches in the small intestine in response to oral immunization or the tonsils and adenoids in the nasal cavity upon nasal or sublingual immunization (3). Further, numerous small isolated lymphoid follicles in the small and large intestine, respiratory tract and the mesenteric lymph nodes as well as the appendix and the colon patches are considered as immune inductive sites. The immune effector sites including the lamina propria and intra-epithelial area of the mucosal surfaces host homing B and T cells generated upon Ag encounter in the
MALT (5, 6). Mucosal tissues involved in immune defence to airborne pathogens are the nasopharynx-associated lymphoid tissue (NALT) and bronchus-associated lymphoid tissue (BALT), found in the upper and lower respiratory tract, respectively (6). NALT is found on both sides of the nasopharyngeal duct in rodents and has been considered analogous to the human Waldeyer’s ring, the lymphoid tissue located in the pharynx and to the back of the oral cavity in humans (7, 8). BALT was first described in the lungs of rabbits but it is largely absent in healthy humans (9). However, BALT formation can be induced in humans in response to microbial exposure or by chronic inflammation as is the case in asthma (10). The organization of MALT has much resemblance to the systemic lymphoid tissues thus including all the immunocompetent cells required to mount an effective immune response i.e. dendritic cells (DC), macrophages and distinct B- and T- cell areas. A unique cell type of the MALT is the microfold cell (M cell) that is found both in the Peyer’s patches and in the crypts of tonsils and adenoids. M cells can ingest pathogens by phagocytosis and pass them on to the underlying antigen presenting cells (11). Interaction of T- and B- cells with mucosal DCs induces their expression of mucosal homing receptors enabling them to home to the original mucosal surfaces and even to those different from the site of infection. This functional connectivity between different mucosal sites was in the early days of mucosal immunology termed ‘the common mucosal immune system’ (12). However, later it has become clear that the mucosal immune system seems to be specially compartmentalized so that e.g. intranasal (i.n.) immunization induces Ag specific immunity in respiratory and reproductive tissues whereas oral immunization predominantly elicits protection in the gastrointestinal tissues [reviewed in (13)]. Chemokine receptor 10 (CCR10) is commonly expressed by most IgA antibody secreting cells (ASCs) and similarly it’s ligand CCL28 is produced by epithelial cells in various mucosal tissues indicating that this is the link between different mucosal sites (14, 15). Homing of IgA ASCs to the respiratory and genourinary tracts following i.n. immunization appears to be further directed by co-expression of CCR10 and α4β1 integrin on the IgA ASCs that bind to CCL28 and VCAM1, respectively (16). Another example of such compartmentalization is immunological connection between the lower gastrointestinal tract and female genital tract. Thus, rectal immunization was shown to elicit immunity in the female genital tract (17). Further, specific targeting of an orally delivered nanoparticle-based vaccine to the large intestine formulated in a pH-dependent microparticle has recently been shown to induce protective immunity in both rectal and vaginal tracts (18).

Dimeric secretory IgA (sIgA) is the dominant isotype at mucosal surfaces with the main function of being neutralizing antibody (Ab) and thereby preventing
the adhesion of pathogens to the epithelial surfaces lining the mucosal surfaces. Dimeric IgA Ab is transported to mucosal surfaces by specific binding to the polymeric immunoglobulin receptor (pIgR) that is expressed on the baso-lateral surface of mucosal epithelium. A part of the pIgR called secretory component (SC) is retained upon release of IgA Ab to mucosal surfaces and may help to protect it against degradation and anchor it to the mucosal layer (19, 20). Interleukin 17 (IL-17) secreted by Th17 cells have been linked to protective mucosal responses against extracellular bacteria, including: S.pneumoniae (21) but more recently it has also been shown to play a role in driving Th1 responses upon BCG vaccination (22). Although much of the protective role of Th17 have been linked to its impact on innate immune responses such as recruitment of neutrophils and macrophages (23), IL-17 has also been reported to up-regulate rapidly pIgR expression in the airway epithelium suggesting its crucial function in IgA transportation to mucosal surfaces (24). Interestingly, transforming growth factor β (TGFβ) that plays an important role for in vivo IgA isotype switching is required for Th17 differentiation (25, 26). Pentameric IgM Ab is also transported through epithelial cells by pIgR whereas IgG Ab is transported by neonatal Fc receptor (27).

MUCOSAL IMMUNIZATION AND VACCINES WITH EMPHASIS ON RESPIRATORY TRACT IMMUNIZATION

A successful mucosal immunization can lead to an enhanced mucosal resistance to infection through various mechanisms, including the induction of Ag-specific sIgA Ab production and, distribution of specific effector T cells in the mucosal tissues as well as alteration in the production of several secretory defense molecules such as mucins, antimicrobial peptides and cytokines (3, 28). However, the mucosal immune system displays a great deal of anatomic compartmentalization related to the migratory profiles of lymphocytes activated at different mucosal sites, which in turn introduce limitations in the selection of immunization route. Thus, mucosal immunization preferentially confers immunity at the directly vaccine-exposed mucosa followed by the adjacent mucosal tissues. In addition, immunization at one mucosal inductive site may also confer an immune response at remote mucosal tissues examples of which are nasal, sublingual and rectal immunization that can lead to induction of immune responses in the mucosa of gastrointestinal/respiratory/genital, gastrointestinal/genital and rectal/genital tracts, respectively (29).
In addition, mucosal immunization can mount a systemic immunity that can counter pathogens that escaped the mucosal immunity. Mucosal immunization may also furnish other advantages over the parenteral vaccination, including increased compliance that may increase the vaccine coverage, as well as reduced risk of transmission of blood borne pathogens as is a potential concern with contaminated injection needles (2). Despite the great promise offered by mucosal immunization, very few mucosal vaccines are currently licensed for human use. These include oral polio vaccines, oral live-attenuated typhoid vaccine, oral cholera vaccines, oral live attenuated rotavirus vaccines, as well as nasal live attenuated flu vaccines (Table 6.1.1). Several issues have been indicated as roadblocks to the development of new mucosal vaccines. Despite significant progress, our understanding of mucosal immune system and how to induce mucosal immunity remains largely incomplete. It has so far proven difficult to mount potent mucosal immunity by mucosal administration of protein/subunit antigens, which is mainly due to the lack of safe and potent mucosal adjuvants and delivery systems. Further, recovery of and functional assays for mucosal antibodies and T cells are labor intensive and technically challenging (1, 30). Given the scope of this book, this chapter will briefly discuss respiratory tract immunization and vaccines.

### Table 6.1.1

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Pathogen involved</th>
<th>Vaccine content</th>
<th>Route of immunization</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RotaTeq (Merk)</td>
<td>Rotavirus</td>
<td>Live-attenuated, monovalent (Rotarix) and pentavalent (RotaTeq)</td>
<td>Oral</td>
<td>(43, 44)</td>
</tr>
<tr>
<td>Rotarix (GlaxoSmithKline)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OPV (Aventis Pasteur India Ltd)</td>
<td></td>
<td></td>
<td>Oral</td>
<td>(45, 46)</td>
</tr>
<tr>
<td>Polio Saven (GlaxoSmithKline)</td>
<td>Poliovirus</td>
<td>Live-attenuated, mono-, bi- and trivalent.</td>
<td>Oral</td>
<td>(45, 46)</td>
</tr>
<tr>
<td>and many more</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dukoral (Crucell)</td>
<td>Vibrio cholerae</td>
<td>Inactivated whole bacteria (Dukoral contains also CTB)</td>
<td>Oral</td>
<td>(47, 48)</td>
</tr>
<tr>
<td>ORC-Vax (VaBiotech)/</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shankol (Shanta Biotechnics)</td>
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Intranasal immunization can target the NALT, which is an important inductive site for the generation of humoral and cellular immune responses to inhaled antigens in the respiratory tract mucosa (1, 12). Inhaled antigens (derived from live attenuated pathogen or recombinant antigen plus adjuvant) are taken up by the follicle-associated epithelium of the NALT, where dendritic cells prime naive T cells. Activated CD4+ T cells differentiate into T helper 1 (TH1), TH2, TH17 cells, regulatory T cells and T follicular helper (TFH) cells. TFH cells contribute to the development of long-lived plasma cells and memory B cells through the germinal centre reaction. Memory B cells and T cells migrate to the draining lymph nodes as well as the effector tissues of the respiratory tract and the genital tract where they exert their protective functions (31).

Currently, there are two nasal flu vaccines licensed for human use. FluMist® (MedImmune) is a live attenuated trivalent vaccine for the prevention of influenza disease caused by influenza A subtype viruses and the type B virus contained in the vaccine: and NASOVAC® (Serum Institute of India) a live attenuated monovalent H1N1 for pandemic flu (32, 33).

Although nasal route of immunization showed promise in the context of live attenuated vaccines and is an attractive approach due to its Ag dose sparing property, which is important in production of massive vaccine doses in the case of pandemic threats (34), there is currently no effective non-living nasal vaccine for human use owing to the lack of safe and effective nasal adjuvants.

Nasal immunization offers several biological advantages over oral immunization. Thus, the nasal mucosa is lined by a specialised epithelium containing numerous antigen-sampling DCs, which extend their dendrites between epithelial cells to reach the lumen. Further, human NALT possesses Ag-retaining crypts, which are absent in the GALT structures such as Peyer’s patches. Furthermore, as opposed to the gastrointestinal tract where Ags are attacked by degrading enzymes, soluble

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</thead>
<tbody>
<tr>
<td>Vivotif (Crucell)</td>
<td><em>Salmonella Typhi</em></td>
<td>Live-attenuated, monovalent</td>
<td>Oral</td>
<td>(49, 50)</td>
</tr>
<tr>
<td>FluMist (MedImmune)</td>
<td></td>
<td>Live-attenuated, trivalent (FluMist) and monovalent (NASOVAC)</td>
<td>Intranasal</td>
<td>(32, 33)</td>
</tr>
<tr>
<td>NASOVAC (Serum institute of India)</td>
<td>Influenza virus</td>
<td></td>
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</table>
Ags are not degraded in the respiratory tract. Animal studies have indicated that much lower dose of non-living Ag is needed for nasal immunization when an appropriate adjuvant is used compared to oral immunization. In addition, the nasal route of immunisation appears to induce a more potent systemic immunity than the oral route (35). It has also been shown in mice that immuno-senescence associated with diminished immune responsiveness occur faster in GALT than in NALT (36). Should this later finding be confirmed in humans, nasal immunization may potentially offer an unparalleled opportunity to immunize elderly individuals.

Notwithstanding, nasal immunization is associated with potential safety concerns owing to the close anatomical connection of the nostrils and olfactory bulbs. Several cases of facial nerve paralysis were reported following nasal administration of exploratory vaccines containing heat labile enterotoxin (LT) and the detoxified LT adjuvant LTK63 (37, 38). While the use of toxin derived adjuvants is not recommended for nasal route (unless verified as safe and well tolerated), cautious should be exercised in the use of other immunostimulatory adjuvants that may potentially trigger a state of inflammation in the human olfactory bulbs following nasal administration.

Pulmonary administration of vaccines is considered to evoke immune responses in the lower respiratory tract induced by BALT. Vaccine formulations intended for the pulmonary route can either be in the form of liquid aerosols or dry powder aerosols. Careful selection of different inhalation delivery systems and aerodynamic particle size can make it possible to target different parts of the lung [reviewed in (39)]. Since the lung is the primary site of infection for MTB the pulmonary route could be especially attractive for novel tuberculosis (TB) vaccines. For example, pulmonary delivery of Ag85B conjugated to Pluronic-stabilized polypropylene sulphide nanoparticles mixed with CpG ODN led to substantial reduction in lung bacterial burden. Interestingly, the enhanced protection was only observed following pulmonary, but not intradermal, delivery of the vaccine highlighting the importance of targeting the vaccine to the site of infection (40). Pulmonary vaccination against measles has been extensively studied in humans. Several reports indicate that aerosol delivery of measles vaccine can induce stronger and more long lasting Ab responses than injected vaccine (41, 42).

In summary, successful introduction of two human flu vaccines given nasally has offered new hopes to develop mucosal vaccines given through intranasal and intrapulmonary routes to engender protective immunity to other respiratory tract infections, including TB.
REFERENCES


