

SUBLINGUAL DELIVERY OF VACCINES

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SUMMARY

Sublingual immunization, where the vaccine is placed directly under the tongue and absorbed through the sublingual mucosa, has the advantages of ease of delivery without the complication of oral administration or the safety concerns of intranasal vaccines. Sublingual vaccine delivery has been shown to induce development of humoral and cell mediated immunity in both the systemic and mucosal compartments to a variety of bacterial, viral, and protozoan antigens. Sublingual immunization represents an exciting breakthrough in vaccine delivery with great potential to overcome the barriers to effective mucosal immunization; this strategy holds great promise specially for vaccinating infants and children in developing countries.

INTRODUCTION

A great deal of effort is being directed towards developing non-parenteral (needle-free) alternatives to traditional vaccine delivery. Non-parenteral vaccines offer a number of potential advantages over traditional vaccines including:

- 1) the ability to confer mucosal as well as systemic immunity,
- 2) increased stability,
- 3) increased shelf-life,
- 4) elimination of needles and the need for specially trained healthcare workers to administer vaccines, and
- 5) lower costs.

Nasal delivery of vaccines has demonstrated efficacy in numerous animal models and in humans (e.g., FluMist®) but there are safety concerns regarding some antigens and adjuvants administered intranasally (Lewis et al., 2009). Transcutaneous delivery (e.g., by skin patch) has shown potential but requires

large amounts of antigen and is device-dependent (Glenn et al., 2007; Frerichs et al., 2008). Oral immunization is effective for some live-attenuated vaccines (e.g., poliovirus, rotavirus, typhoid fever) and killed whole-cell vaccines (e.g., cholera). However, oral immunization with non-replicating vaccines requires large antigen doses and some means to bypass or neutralize the gastric acidity; furthermore, the outcome of oral vaccination may be impacted by the age and immune status of the host, the presence of maternal antibody in infants, colonization and growth of microbial flora in the bowel, and numerous other factors.

The sublingual mucosa has been a site for administration of therapeutic drugs for over a century. This tissue is highly vascularized, which allows rapid entry of antigens into the systemic circulation and avoidance of the harsh pH

and proteases found in the gastrointestinal tract and first-pass metabolism of the liver (Zhang et al., 2002). As with sublingual drug delivery, the ease of administration, safety benefits and increased patient compliance also make this route attractive for vaccine delivery. Sublingual immunization (SLI) is accomplished by placing the immunizing preparation directly under the

tongue where it is absorbed through the sublingual mucosa. The presence of immune sentinel cells within the oral mucosa makes it a suitable site for direct antigen uptake and initiation of an immune response. Moreover, SLI induces immune responses systemically as well as in distal mucosal compartments, making this a promising delivery route for mucosal protection.

ANTIGEN PERMEATION, UPTAKE AND PROCESSING WITHIN THE SUBLINGUAL MUCOSA

Different compartments of the oral mucosa, specifically the lining, masticatory, and lingual and sublingual mucosa, exhibit different structural, immunological and chemical properties, and consequently have different permeabilities to exogenously applied proteins and other substances. The sublingual mucosa of humans is covered with a non-keratinized stratified squamous epithelial layer. As a result, this surface is more permeable than other regions of the oral mucosa, such as the gingiva and hard palate, which are keratinized and more closely resemble the epidermis of the skin (Squier, 1991). However, permeation does not occur freely, since the presence of extruded amorphous intercellular lipids provide some barrier to chemical permeation in the supra-basal sublingual mucosa; and salivary mucins also inhibit permeability of some substances at the sublingual surface (Squier, 1991).

In marked contrast to humans, rodents have a highly keratinized sublingual mucosa; but despite this difference, mice are suitable models for pre-clinical evaluation of sublingual vaccines. Using this animal model, it has been observed that small volumes of sublingually applied antigens stay localized and do not “spread” to other

tissues. When fluorescently-labelled ovalbumin (OVA) was administered sublingually in a small volume (5-10 μ l) to mice, the antigen remained exclusively on the sublingual mucosa for up to two hours. No fluorescent OVA was detected in the buccal tissues, palate, oesophagus, or small intestine, indicating that initiation of the immune response occurs specifically within the sublingual mucosa. When larger volumes were sublingually applied, some OVA was swallowed and detected in the oesophagus and duodenum (Çuburu et al., 2007).

Resident and migratory antigen-presenting cells (APCs) of the sublingual mucosa are directly involved in antigen transport to the draining lymph nodes. The sublingual lamina propria of naive mice contains a wide distribution of leukocytes, including MHC-II⁺ cells, located mainly along and beneath the basal layer of the epithelium. Almost all of these are CD11c⁺ dendritic cells, and represent 3-4% of all cells in the sublingual compartment. Within 2 hours of sublingual administration of cholera toxin (CT), the number of MHC-II⁺ dendritic cells in the submucosa and epithelium increased, indicating a rapid recruitment to the site of administration (Çuburu et al., 2007; Song et al., 2009); the dendritic cell

numbers then returned to basal levels 6 hours post-treatment (Çuburu et al., 2007).

Typically associated with antigen uptake and presentation in the skin epidermis, Langerhans cells represent a small population of cells interspersed in the human sublingual epithelia (Allam et al., 2008). In mice, the presence of langerin⁺ cells, a hallmark of Langerhans cells, has also been noted in naive animals (Çuburu et al., 2007; Song et al., 2009; Hervouet et al., 2010). Depletion of this cell population had no impact on CD4 T-cell proliferation in draining lymph nodes after SLI in the murine model, suggesting that these cells are not essential for activation of CD4 T-cells (Song et al., 2009). However, sublingual langerin⁺ cells did induce proliferation of CD8 T-cells, indicating that these cells are functional APCs. Their role is distinct from langerin⁻ dendritic cells, which were effective in inducing both CD4 and

CD8 T-cell proliferation (Hervouet et al., 2010). Dendritic cells of the sublingual tissue prime B- and T-cells in the draining lymph nodes via the CCR7-CCL19/CCL21 pathway (Song et al., 2009). This pathway has similarly been shown to regulate skin dendritic cell entry into the dermal lymphatics (Ohl et al., 2004). A role of resident macrophages in shaping the immune response to sublingually applied antigens has also been reported (Jee et al., 2010). Within the human sublingual mucosa, mast cells localizing in the gingiva along basal cells and within lobes and ducts of glands are also known to participate in the ensuing immune responses (Allam et al., 2008). It is likely that other cell populations of the sublingual mucosa have some impact on the immune response to antigens and adjuvants; however, the direct or indirect role of other cell populations is not yet fully understood.

IMMUNE RESPONSE TO SUBLINGUALLY-ADMINISTERED ANTIGEN

Humoral response to sublingual immunization

The ability of sublingual immunization to induce a humoral immune response systemically as well as in multiple mucosal compartments has been demonstrated with a variety of viral, bacterial, and protozoan antigens. In the murine model with OVA in the presence of CT as a mucosal adjuvant, a significant systemic antibody response was observed upon sublingual administration, as shown in Figure 1. The response to sublingually-applied OVA was similar in magnitude and isotype distribution to the response seen with intranasal immunization and both sublingual and intranasal responses were enhanced when compared with intragastric administration (Çub-

uru et al., 2007). BenMohamed and co-workers (BenMohamed et al., 2002) also noted a strong systemic immune response upon SLI with synthetic lipopeptides derived from *Plasmodium falciparum*; importantly these antibodies were able to recognize and bind intact parasites.

Antibody-production in the gastrointestinal tract has also been noted in response to SLI. Negri and co-workers noted detectable salivary IgA up to four months post-immunization (Negri et al., 2010). Likewise, Song and co-workers detected significant IgA in saliva and faecal extracts of mice (Song et al., 2008). This suggests the suitability of SLI for vaccines against enteric pathogens that primarily colonize the gastrointestinal epithelium.

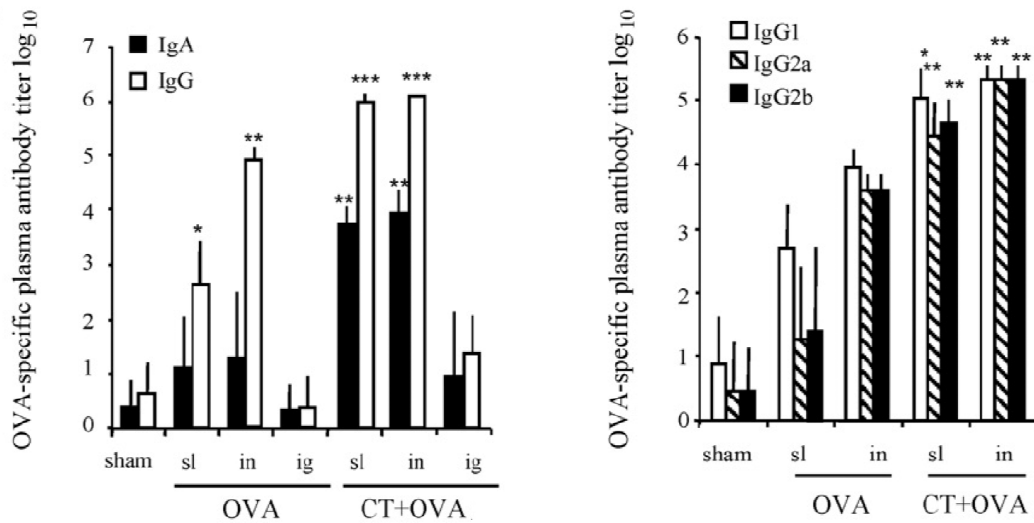


Figure 1: Systemic antibody levels upon sublingual (sl), intranasal (in), and intragastric (ig) immunization with ovalbumin and cholera toxin (CT). From *Çuburu et al. 2007*; with permission.

Generation of antibody responses in the genital tract is also an important aspect of SLI and suggests that this route of immunization could be promising for vaccines against sexually transmitted diseases, as well. Sublingual administration of a human papillomavirus antigen (HPV16L1) induced higher vaginal secretory IgA than intravaginal administration of a larger dose of the same antigen. Intranasal administration also elicited significant vaginal secretory IgA, but saliva antibody production was lower than with sublingual delivery (*Cho et al., 2010*). In a recent investigation, sublingual administration of an HIV-1 ectodomain protein was shown to induce antigen-specific IgG and IgA in genital secretions at levels comparable to levels induced by local intravaginal immunization (*Hervouet et al., 2010*).

In the respiratory tract, antigen-specific antibodies were detected following SLI with OVA. Significant OVA-specific IgG and IgA titres were detected in the nasal wash and broncho-alveolar lavage of immunized mice

(*Çuburu et al., 2007*). Immunization with inactivated influenza virus also yielded significant anti-viral IgA and IgG in lung and nasal wash fluids (*Song et al., 2008*).

The origin of antibodies produced in response to SLI has been assessed to better understand the response to sublingual vaccination. Upon SLI with inactivated influenza virus administered in conjunction with a chimeric mucosal adjuvant composed of the B subunit of LT and the A subunit of CT (mCTA/LTB), IgA antibody-secreting cells were detected in various mucosal tissues, including the lung, nasal passage, submandibular glands, and small and large intestine, in addition to the spleen (Figure 2) (*Song et al., 2008*). *Çuburu* and co-workers detected IgG- and IgA-secreting cells in the lung, spleen and submandibular lymph nodes but not in the mesenteric lymph nodes following SLI (*Çuburu et al., 2007*). Antibody-secreting cells in the genital tract were also noted in response to SLI with an HIV antigen. While antigen-specific antibody-secreting cells were

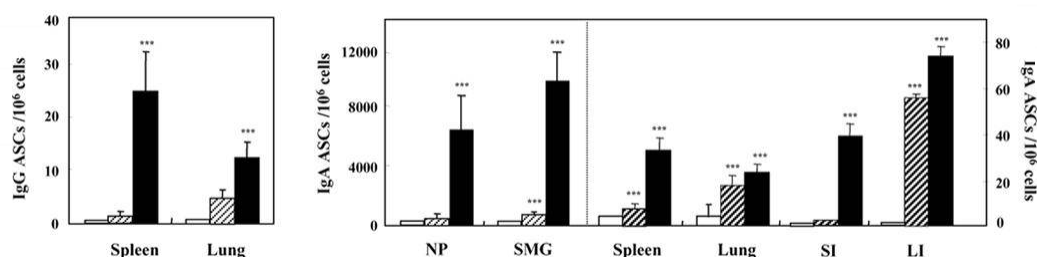


Figure 2: IgG and IgA antibody-secreting cells were detected in the spleen and mucosal compartments of mice treated sublingually with PBS (open bar), inactivated A/PR/8 influenza virus (hatched bars), or inactivated virus plus a mutant chimera of the B subunit of LT and the A subunit of CT (mCTA/LTB) (closed bars). Antibody-secreting cells were measured in the spleen, lung, nasal passage (NP), submandibular gland (SMG), small intestine (SI), and large intestine (LI). From *Song et al., 2008*; with permission.

detected in the genital tract of sublingually immunized mice, none were detected in the ileosacral lymph nodes, which drain the genital mucosa. This suggests that antibody-secreting cells migrated to the genital tract from another location, most likely the draining lymph nodes of the sublingual mucosa (*Hervouet et al., 2010*). CCL28 was shown to be an important chemokine in migration of IgA antibody-secreting cells to the genital tissue upon SLI (*Çuburu et al., 2009*). Indicating the duration of antibody production, antigen-specific antibody-secreting cells were found in the bone marrow of mice immunized with tetanus-toxoid and appropriate adjuvants up to four months post-immunization (*Negri et al., 2010*).

Cell-mediated responses to sublingual immunization

Several investigators have characterized the cell-mediated immune response to sublingually administered antigens. Although the quality of the Th1/Th2 response is influenced by both the antigen and the adjuvant, investigations of CD4 T-cell responses have noted a mixed Th1/Th2 response following SLI with various formulations. This was noted in the spleen and sub-

mandibular lymph nodes following SLI (*Çuburu et al., 2007; Çuburu et al., 2009; Zhang et al., 2009; Cho et al., 2010*). The development of CD8 cytotoxic lymphocytes in distal mucosal compartments has been noted as well. *Hervouet et al.* observed IFN- γ production and cytolytic activity of CD8 cells in the genital tract of mice immunized sublingually with an HIV reverse transcriptase peptide conjugated to the binding subunit of cholera toxin (CT-B) (*Hervouet et al., 2010*). Cytotoxic T cell activity was also detected in the lung following SLI with ovalbumin (Figure 3) (*Çuburu et al., 2007*). CD4 and CD8 T-cell expansion in response to antigen re-stimulation was noted in the spleen, submandibular (regional) lymph nodes, and distal lymph nodes draining the genital mucosa (iliac lymph nodes) four months post-immunization (*Negri et al., 2010*).

Adjuvant incorporation in sublingual vaccines

The incorporation of appropriate adjuvants into sublingual vaccines is critical and enhances the immune responses in many instances (e.g., [*Çuburu et al., 2007, 2009; Song et al., 2008*]). The most commonly used adjuvants in SLI are derivatives of the

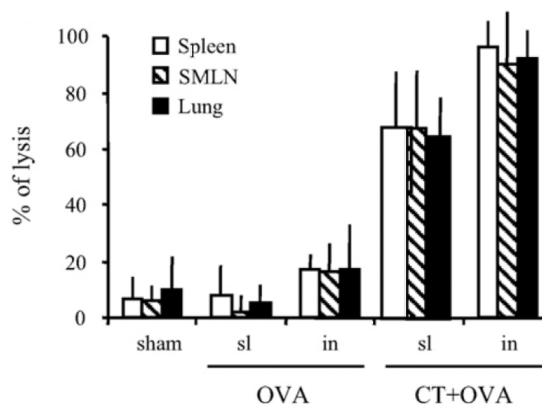


Figure 3: Sublingual (sl) and intranasal (in) immunization with ovalbumin (OVA) with or without cholera toxin (CT) induced *in vivo* cytotoxic activity in the spleen, submandibular lymph node (SMLN), and lung. From Çuburu et al., 2007; with permission.

bacterial ADP-ribosylating enterotoxins, CT and LT. In a direct comparison of several adjuvants co-administered with a human papillomavirus protein 16 L1, only the B subunit of CT significantly enhanced mucosal and systemic immune responses. Other adjuvants included various TLR agonists, NOD agonists, vitamin D₃, and nanoparticles of a bacterial capsular exopolymer (Cho et al., 2010). CpG ODNs have also been successfully used in a sublingual *Salmonella* vaccine delivered to neonatal mice (Huang et al.,

2008). Negri and co-workers noted the impact of LT-derived adjuvants in eliciting long-lasting systemic and mucosal immune responses (Negri et al., 2010).

Protective efficacy of sublingual immunization

In vivo challenge studies in mice have shown the protective efficacy of SLI against a variety of pathogens. Incorporation of mCTA/LTB with an inactivated whole influenza virus induced complete viral clearance in the

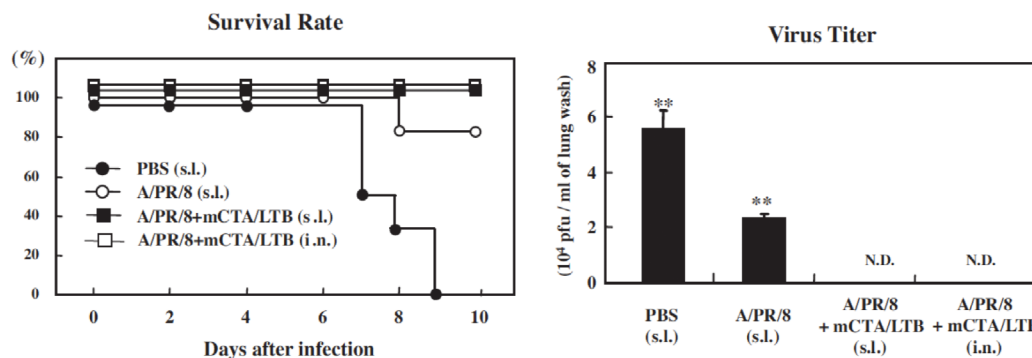


Figure 4: Sublingual immunization with adjuvant conferred complete protection against intranasal viral challenge and resulted in no detectable viral titres in the lung. Mice were immunized sublingually (sl) or intranasally (in) with inactivated A/PR/8 influenza virus with or without a mutant chimera of the B subunit of LT and the A subunit of CT (mCTA/LTB). From Song et al., 2008; with permission.

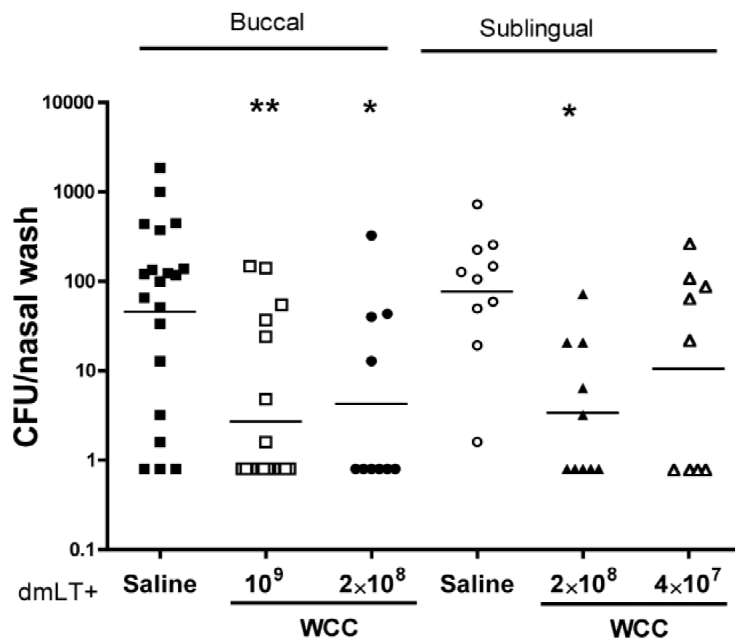


Figure 5: Dose-dependent protection was induced following sublingual and buccal immunization with killed pneumococcal whole-cell vaccine containing a detoxified double mutant of LT (dmLT). Mice were immunized three times and challenged intranasally one week following the last immunization with the indicated dose. Determination of CFU per nasal wash was performed 1 week post-challenge. From *Lu et al., 2010*; with permission.

lungs and 100 percent protection following homologous intranasal viral challenge; without adjuvant, mice had significant viral titre in the lungs 3 days post-challenge and only partial protection (80% survival) as seen in Figure 4 (*Song et al., 2008*). SLI with human papillomavirus virus-like particles resulted in complete protection against genital challenge with papillomavirus pseudovirions (*Çuburu et al., 2009*). Protection against *Porphyromonas gingivalis* was observed in mice immu-

nized sublingually with an outer membrane protein of this organism plus a cDNA vector plasmid encoding a Flt ligand; protection was noted by reduced bone loss following oral challenge (*Zhang et al., 2009*). A detoxified mutant of LT (dmLT) developed in our own laboratory and administered as part of a whole-cell killed pneumococcal vaccine sublingually also conferred significant protection against intranasal challenge as seen in Figure 5 (*Lu et al., 2010*).

SAFETY OF SUBLINGUAL IMMUNIZATION

Several studies have addressed issues related to safety of administering vaccine formulations sublingually because of the potential risk associated with trafficking of intranasally administered

antigens to the central nervous system. Live influenza virus administered sublingually in mice was undetectable in the olfactory bulb or brain tissue 24-hours post-inoculation; however, there

was detectable viral RNA and labelled virus in these compartments following intranasal administration (Çuburu et al., 2007; Song et al., 2008). Additionally, administration of CT-B sublin-

gually resulted in no adverse events or other side effects that have been noted upon intranasal administration (Cho et al., 2010).

CONCLUSION

Despite the availability of antibiotics and vaccines, infectious diseases remain a leading cause of morbidity and mortality worldwide, especially in developing countries. Even when vaccines exist, they are often impractical, especially for administration to children. New strategies for vaccine discovery, formulation and delivery are desperately needed, and successful development of vaccines that are safe, well tolerated and effective will have a profound impact in improving health globally. Oral vaccines are potentially advantageous but must overcome a number of physical and physiological barriers (e.g., gastric acidity, age and immune status of the host, maternal antibody in infants and bowel microbial flora) to be effective. Sublingual

immunization, where the vaccine is placed directly under the tongue and absorbed through the sublingual mucosa, has the advantages of ease of delivery without the complication of oral administration or the safety concerns of intranasal vaccines. Sublingual vaccine delivery has been shown to induce development of humoral and cell mediated immunity in both the systemic and mucosal compartments to a variety of bacterial, viral, and protozoan antigens. Sublingual immunization represents an exciting breakthrough in vaccine delivery with great potential to overcome the barriers to effective mucosal immunization; this strategy holds great promise specially for vaccinating infants and children in developing countries.

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